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Hypothesis: repletion of mild zinc (Zn) and iron (Fe) deficiencies will improve neuropsychological functions of women. Design: randomized double-blind trial of Zn and Fe repletion with a cross-over. 60 Zn-Fe deficient and 20 normal subjects are studied. Main Outcomes: changes in neuropsychological functions; relationships between neuropsychological functions and Zn kinetics, Zn and Fe status, body composition and metabolism. Results: 158 women phone screened; 61 detailed screened; Zn kinetics done in 14; and 6 treated; Zn kinetics done in 5 men; the 24-h model was concordant with the 9-d triexponential model; the R^2 of the 24-h model fitted to the ^{67}Zn disappearance data was 0.993; the 24-h plasma Zn pool by analysis of plasma 24-h after ^{67}Zn dose = "truncated" rapidly exchangeable Zn pool (EZP); the "truncated" EZP was systematically 20% > the 9-d mammillary model (which accounts for zinc losses and quasi-equilibrium); the "truncated" EZP was highly correlated with body weight (r= 0.925), lean body weight (r= 0.861), and creatinine excretion (r= 0.900); the urinary Zn elimination constant was highly correlated with plasma Zn (r= 0.785); the 24 hour spot urine EZP was about 20% less than the 24 hour spot plasma EZP (r= 0.911).

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PI - Signature 11/22/96 Date

Table of Contents

(5) Introduction

Subject: This project investigates the relationship of zinc and iron nutriture to human neuropsychological function.

Purpose: This project tests the hypothesis: "Repletion of mild zinc and iron deficiencies will improve neuropsychological (neuromotor and cognitive) functions of premenopausal women."

Scope: The project has three major components:

- Women at risk of mild Zn and Fe deficiencies are identified through food frequency histories. This approach was effective in a previous project that measured the relation between zinc and iron nutriture of young women (1).
- The zinc and iron status of the subjects are characterized by techniques (zinc kinetics, serum ferritin, hemoglobin and red blood cell indices) that were effective in a previous project that measured the relation between zinc and iron nutriture of young women (1).
- Changes in neuropsychological function after zinc and iron repletion are measured by computerized neuropsychological tasks (adapted by Co-Investigator Penland) using a double-blind randomized controlled treatment cross-over design, with deficient and normal control groups. Our previous findings provided the basis for this project (2-5).

(6) Body

Statement of Work

This 3 year project tests the hypothesis: "Repletion of mild zinc and iron deficiencies will improve neuropsychological (neuromotor and cognitive) functions of premenopausal women."

The design is a double-blind randomized controlled treatment trial with a cross over of treatments. Sixty zinc and iron deficient, and 20 normal control subjects are studied. Twenty of the deficient subjects and the 20 normal subjects are given placebo to control for study effects. Neuropsychological functions are measured at baseline and after 8 and 16 weeks of treatment. The cross-over is at 8 weeks. The placebo administered subjects undergo a pseudo-cross over at 8 weeks. The main outcome is the change in neuropsychological functions subsequent to treatment. The individual and combined effects of zinc and iron treatment, and unique effects related to the order of treatments are determined. Secondary outcomes include comparison of methods for measurement of rapidly exchangeable zinc pools; comparison of kinetic indices to other indicators of zinc status; measurement of relations between indices of zinc kinetics to other indicators of metabolism; measurement of relations between zinc kinetics to various indicators of iron status; and measurement of relations between indices of zinc and iron status to food frequency.

Project Time Line (from the grant application, with comments):

0-90 days
- Recruit, interview and hire the physicians assistant and masters level technician
- Purchase initial equipment and supplies including:
  - Computer for neuropsychological testing
  - Treatments
  - ^{67}Zn tracer for measurements of zinc kinetics
- Prepare ^{67}Zn tracer for human administration
- Advertise project
- Start screening respondents to advertisements

Year 1. Enroll 30 subjects in treatment trial, measure cognition (**Note**. We encountered difficulty with recruitment and are behind schedule)
Year 2 Enroll 30 subjects in treatment trial, measure cognition
Year 3 Enroll 20 subjects in treatment trial, measure cognition
Prepare final reports for publication

Subjects Time Line (from the grant application, with additions):

d 1 Telephone interview respondents to advertisements
d 7 Screen respondent (n=150) to qualify for the project (**Note**. Experience this year has shown we will need to screen as many as 500 women. We are using resources of the NIH sponsored Clinical Research Center for screening.)
Medical history
Food frequency history
Physical Examination
Screening blood chemistries, urine and fecal examinations
Serum ferritin, hemoglobin, RBC indices and sedimentation rate
d 14 Select subject (n=100) based on iron criteria
Start treatment of latent deficiencies with micronutrients
Schedule admission to the Clinical Research Center
d 21 Admit to Clinical Research Center for zinc and iron status (**Note**. These procedures are done on an out patient basis when subject has time constraints)
1700 regular supper
1800 begin overnight fast
d 22 0645 begin measurements of zinc kinetics
insert catheters into both antecubital veins
0715 start 24 hour urine collection for total zinc
0718 draw baseline blood for zinc and iron indices
0720 inject 1 mg 67-zinc intravenously
0740 take blood for zinc disappearance and turnover rate (**Note**. The collection times are modified to increase data)
Later orient to computerized neuropsychological tasks
d 23 0715 end 24 hour urine collection and take blood for exchangeable zinc pool (**Note**. Additional blood has been collected for measurements of zinc kinetics)
begin 60 minute urine collection for exchangeable zinc pool (**Note.** Additional urine has been collected in some subjects, for measurements of zinc kinetics)
0900 discharge with instructions for next phase
d 35 Select subjects (n=80) based on zinc and iron criteria.
Randomize subjects to the treatments
micronutrients (placebo) - 20 normal and 20 deficient subjects
micronutrients (placebo) with 30 mg zinc - 20 deficient subjects
micronutrients (placebo) with 30 mg iron - 20 deficient subjects
d 40± Measure baseline neuropsychological functions on day 8-12 of menstrual cycle (**Note**. Measure Bioelectrical Impedance and collect blood for add-on studies)
Begin treatment
d 96 After 8 weeks of treatment measure follow-up neuropsychological functions on day 8-12 of menstrual cycle
(**Note**. Measure Bioelectrical Impedance and collect blood for add-on studies indicated below)
Cross-over the zinc and iron treatment groups and pseudo-cross-over the placebo groups

d 152 After 16 weeks of treatment measure neuropsychological functions on day 8-12 of menstrual cycle
(**Note**. Measure Bioelectrical Impedance and collect blood for add-on studies indicated below)
Thank subjects for participation
Give subjects copy of their medical record
Give subjects nutrition information re: zinc and iron
Pay the final compensation to the subjects
Later Send subjects copies of published reports

Methods

Institutional Review. The study was approved by The University of Texas Medical Branch Institutional Review Board, and the USDA, ARS Human Studies Review Committee.

Informed Consent. The informed consent statement was modified to meet requirements of the US Army. Participants give written consent after being informed in laymen's terms both verbally and in writing of the purpose, procedures, risks and benefits of the study.

Publicity. The study is advertised on the University bulletin boards, in the University News Paper, the Galveston Daily News and the Houston Chronicle.

Eligibility. To increase the number of potential subjects the age range has been increased from 19-35 to 19-40 years.

Inclusion. Ages 19-40 years; regular menses with a cycle of 24-34 days; good health; body mass index ±10 % of desirable; completion of 12 grades of school; good understanding of the information provided during the informed consent process; income $\geq$ 2 x 'poverty'; and normal screening laboratory tests. Ethnicity, religion, sexual preference, and employment will not be factors.

Exclusion. Recurrent or chronic illness; eating disorders; chronic medication; nutritional supplements that contain Fe or Zn in the past 60 days; failure to menstruate the previous month; daily consumption of more than two ounces of ethanol or 10 cigarettes daily; and recreational use of controlled substances.

Telephone Screen. One hundred fifty eight women have contacted us by phone. The project is briefly described and health information elicited. We attempt to identify individuals in good health who are truly interested. Sixty one (37%) individuals met the criteria for further screening. Reasons for rejection include obesity (BMI >29, 15%), chronic disease, chronic medications, or nutritional supplements (11%), irregular menstruation (13%), lack of commitment or time constraints (13%), other reasons (11%)

Detailed Screening (including iron status). Iron status was measured in the sixty one subjects who were screened by medical history, food frequency, and physical examination. Laboratory analysis was done by the UTMB Hospital Clinical Pathology Department. Included were erythrocyte sedimentation rate, complete blood count, erythrocyte indices, serum ferritin, serum iron, percent saturation of transferrin, and automated screening chemistries ('liver enzymes', urea nitrogen, creatinine, uric acid, total cholesterol, glucose, total protein, albumin, sodium, potassium, chloride, calcium, phosphorus), and urinalysis and fecal guiac test for blood. Individuals are rejected from further study for abnormal medical findings, inability to commit the time, and pregnancy.

For this study serum ferritin concentration of $\leq$ 18 ng/mL is designated low. Serum ferritin 30-60 ng/mL is designated normal. Persons with serum ferritin 19-29 ng/mL are excluded. All subjects have normal hemoglobin and RBC indices.

Fourteen subjects were selected for further study (zinc kinetics and neuropsychological function if they meet the zinc status criteria).

Questionnaires. Questionnaires are self administered. Answers are reviewed with the subject by a physician. (See appendix for copies of questionnaires). The food frequency questionnaire is based on that used in NHANES- II.

Treatments. Treatments are micronutrients (M) without zinc or iron, 30 mg zinc encapsulated with M (ZnM), and 30 mg iron encapsulated with M (FeM). The micronutrient preparation, M, serves as placebo. M provides 50 % of the RDA or 50 % of the Estimated Safe and Adequate Daily Dietary Intake of micronutrients listed in the RDA (6), with the following exceptions. Calcium, magnesium, and phosphate are excluded because they are potential inhibitors of zinc and iron retention (7, 8). To minimize potential interference with zinc metabolism folate is included at 25 % of the RDA (9-11).

The treatments are prepared in identical appearing tablets by the General Nutrition Company. The nutrient contents is certified by William Dorrow, the person in charge of the Company's special preparation unit. The zinc and iron contents are confirmed by chemical analysis.

All subjects are treated with micronutrients throughout the experiment, beginning at least 7 days before they participate in measurements of zinc kinetics. Fourteen subjects have been treated with the micronutrient supplement. Thus only effects of low zinc and iron status are being studied.

After the baseline measurements of neuropsychological functions subjects are treated for 8 weeks. After completion of 8 weeks and measurement of functions the ZnM subjects are switched to the FeM and the FeM subjects are switched to the ZnM treatment. All other subjects undergo a pseudo-cross-over to maintain blinding. After cross-over subjects are treated with their respective treatments for 8 weeks. Final measurements of functions are then done.

Compliance. Treatments are administered in batches of 35 capsules. Subjects are interviewed weekly by phone to ascertain progress and the number of capsules remaining. Subjects will be seen monthly and health, diet and the number of capsules remaining determined.

Replacement. Subjects are replaced if they are non-compliant, have $\geq$ 5 days of febrile illness, accidents that cause severe stress, have a substantial change in diet (detected by history), or fail to menstruate.

Coercion. No subject is coerced. There is no penalty for resignation or discontinuance.

Records. Subjects are given a copy of their health data and may have it sent to their personal physician.

Confidentiality. Records are kept in a locked file. Subjects are not be identified as to person.

Compensation. Compensation is proportional to participation.

Zinc Status.

On the day of the zinc kinetics study, venous blood is collected in Sarstadt syringes containing sodium heparin at 07:15 hours after an overnight fast for leukocyte, lymphocyte and platelet zinc and plasma Zn. Blood is kept cool and delivered to the laboratory within the hour. As indicated below (discussion of zinc kinetic findings), in selected subjects blood samples are collected more frequently than originally planned to better define zinc kinetics.

Processing. Leukocytes, lymphocytes and platelets are separated from whole blood by a gradient technique (12). Plasma for analysis of zinc is separated from the cells by centrifugation at 7°C and 2500 x gravity for 10 minutes.

Urine Samples. A 24 hour urine collection for zinc is initiated shortly before injection of the ^{67}Zn tracer. A 1 hour urine is collected for ^{67}Zn at the end of the 24 hour urine collection. In selected subjects additional urine is collected for measurement of ^{67}Zn excretion up to 9 days after administration.

Hair Samples. About 1 gram of hair (the proximal 1 cm) is collected from the occipital scalp using stainless steel scissors. The sample is placed in a 15 mL trace-metal free plastic digestion tube and delivered to the laboratory.

Zinc Status. Plasma, leukocytes, lymphocyte, platelet (13), urine and hair (14) are analyzed by Atomic Absorption Spectroscopy (AAS). Zinc kinetic measurements (1, 15-17) include the 30-60 minute plasma ^{67}Zn disappearance and turnover rate, and the exchangeable zinc pools at various intervals after intravenous injection of the ^{67}Zn.

A plasma zinc disappearance constant (k) ≥ 0.02 min^{-1} designates the low zinc status. A plasma zinc disappearance constant (k) ≤ 0.018 min^{-1} designates normal zinc status. The interval between the disappearance constants prevents overlap. Determination of relationships between zinc kinetic indices to other indicators of zinc status is one of the secondary objectives of the project.

Plasma Zinc Disappearance & Turnover Rate. The 30-60 minute plasma zinc disappearance and turnover rate are measured by inductively coupled plasma-mass spectrometry (ICP-MS) has been described our previous reports (1, 18).

After an overnight fast of at least 12 hours, Teflon catheters are placed in both antecubital veins. One is attached to a slow infusion of 0.5 N saline. The other is filled with heparin and closed with an obturator. Thirty minutes later, 25 mL of blood is taken from the obturated catheter for assay of the natural ^{67}Zn:^{68}Zn ratio in plasma. Immediately after, 2.0 mg ^{67}Zn (sterile, pyrogen free) in 15 mL of 0.5 N saline is rapidly injected over into the saline stream of the infusion catheter. This is followed by the rapid administration of 0.5 N saline for 30 seconds. Blood samples (25 mL) are taken from the obturated catheter at intervals specified below in the discussion of the zinc kinetic studies. Between samples a heparinized obturator is placed in the lumen of the catheter.

Details of the ICP-MS analysis have been reported(18).

Exchangeable Zinc Pool Sizes. The exchangeable zinc pool sizes are measured are described below in the results of the zinc kinetics studies.

Neuropsychological Evaluation. Six subjects have completed the baseline assessment of neuropsychological function and are now receiving experimental treatments.

Measurement of functions is timed to days 8-12 of the menstrual cycle.

The tasks are from a Library compiled by Co-Investigator Penland of the USDA/ARS Human Nutrition Research Center in Grand Forks, ND for use at the Grand Forks Center and at the USDA/ARS Human Nutrition Research Center in San Francisco, CA. They have face validity and known reproducibility.

Subjects are tested by a physician (Egger) trained in test administration. Testing is done after breakfast in a quiet room on the CRC. Subjects are requested to get a good nights rest the night before and not to consume ethanol or other psychoactive substances. Test administration requires 90 minutes. This includes a short rest half-way through the session. Subjects are oriented to the test procedure and content before collection of data. This decreases contamination related to learning and practice. Testing will be done on the 8-12 day of the menstrual cycle.

Neuropsychological data are copied on two backup disks and stored. Data on the computer are transmitted directly to Co-Investigator Penland by the Internet.

Task Library

- **Attention.** Attention is measured by vigilance (19), orienting (20), color-word naming (21), perceptual processing (22) and continuous vigilance (23) tasks.

- **Perception.** Perception is measured by a letter-matching (24), automatization (25), short term memory of characters displayed on a screen (26), and time estimation (27)tasks.

- **Higher Cognitive Processes.** Higher cognitive processes are measure by symbol digit (28), mathematics (29), shape recognition (30), verbal learning (31), and concept formation task (32, 33) tasks.

- **Spatial Orientation.** Spatial orientation is measured by maze (34), and cube recognition (35, 36) tasks.

- **Psychomotor skills**. Psychomotor (sensory-motor) skills are measured by tapping (32, 37), steadiness (29, 38), pursuit (39), trails (37, 40, 41), and direction tasks.

Reports. Findings are reported at national meetings and in the peer reviewed literature. Two posters describing measurements of zinc kinetics were presented in 1996 at national meetings, the Central Society for Clinical Research and the American College of Nutrition (see appendix).

Additions and Changes to the Original Protocol.
- Subject age limit was increased to 40 years, to increase the number of potential participants.
- Subjects are given the option for doing the study as an outpatient. Many potential subjects can not take the time for admission to the CRC.
- Bioelectrical Impedance of fat free mass is measured before and after treatments. The relation of mild zinc and iron deficiencies to body composition is unknown.
- Opioid peptides, serotonin, dopamine and other neurotransmitters in blood are being measured before and after treatment by Sam Baethena, Ph.D. of the USDA/ARS Human Nutrition Research Center, Beltsville, MD (at minimal cost). Mechanisms of zinc's and iron's actions in the CNS are uncertain.
- Blood folic acid, cyanocobalamine, pyridoxine, riboflavin, and homocysteine are being measured by T Tamura, M.D. of the Department of Nutritional Sciences, University of Alabama at Birmingham (at no cost). Zinc has been implicated in metabolism of folate, pyridoxine, homocysteine and possibly riboflavin. This study offers an opportunity to explore these relationships.
- Plasma amino acids are measured before and after treatment by Professor Fritz of UTMB (at minimal cost). Zinc is essential for amino acid utilization. Effects of mild deficiency are unknown.
- Urine and plasma are assayed for ionized calcium, phosphate, osteocalcin, and parathormone before and after treatment by David Simmons, Ph.D. of UTMB (at no cost). Zinc is essential for bone metabolism. Effects of mild zinc deficiency on bone are unknown.
- Activity of cytochrome P-450 is measured before and after treatment by Douglas Goeger, Ph.D. of UTMB in response to oral administration of the substrate chlorzoxazone (at minimal cost). The volunteers are given a tablet containing 500 mg of chlorzoxazone on an empty stomach and blood plasma is essayed for chlorzoxazone metabolites two hours later. Zinc and iron have been implicated in P-450 activity. Effects of mild zinc and iron deficiencies are unknown.
- Taste acuity is measured before and after treatment by the two methods. The electrogustometer involves the application of a low electric current to regions of the tongue to test the threshold response of taste buds. The filter-disc method for measuring the threshold of the taste substances applies the increasing concentrations of sodium chloride (salt), sucrose (sweet), tartaric acid

(sour), and quinine hydrochloride (bitter) to the corresponding region of the tongue. Effects of mild zinc and iron deficiencies on taste acuity are unknown.

- Zinc kinetics have been studied in more detail than originally proposed (see below).

Results and Discussion

1. Zinc content of platelets, lymphocytes and granulocytes is being measured. Published reports (12, 42) suggest these indices are sensitive indicators of zinc status. Data are available from 11 women and 5 men. The results expressed as µg Zn/10^{10} cells (Table 1).

Table 1. Zn Concentration in Platelets, Lymphocytes, Granulocytes and Hair

Subject	Platelet Zn	Lymphocyte Zn	Granulocyte Zn	Hair Zn
	(µg/1010 cells)	(µg/1010 cells)	(µg/1010 cells)	(µg/g)
6	6.9	148.5	146.0	-
7	6.4	103.5	138.9	215.0
9	3.4	113.3	36.5	138.8
8	5.2	59.2	45.5	151.4
10	3.1	35.3	37.0	160.9
12	2.6	40.7	280.0	154.0
11	2.5	273.7	38.8	232.9
13	3.5	135.9	30.6	77.2
16	5.6	88.9	79.2	99.2
14	3.0	109.5	41.1	127.6
15	2.7	140.7	84.7	117.9
reference values	**3.0-6.6 (43)**	**45.0-218.0 (43)**	**37.8-117.0 (43)**	**100-300 (44)**

- Except for three women (#s 12, 11, and 15) the values for platelet zinc were within the reference range (3.0 - 6.6) given by Wang et al (43).
- One man had a very low platelet zinc concentration although the platelet yield was high.
- Lymphocyte zinc was below the lower limit of the reference range (45-218) in 2 women (#s 10, and 12) and elevated in one (# 11).
- In 4 men in which it was determined, lymphocyte zinc was within the reference range.
- Granulocyte zinc was below the reference range (37.8 - 117) in three women (#s 9, 10, and 13); two (#s 7 and 12) had elevated levels.
- The yield of lymphocytes was >93 % in six women. In two where the yield was 66% there was appreciable contribution to the cell count by monocytes. Since the zinc content of monocytes is similar to that of lymphocytes, the zinc content of individual cells is representative of both types.
- The yield of granulocytes was similar to that of lymphocytes. However, in one subject (# 12) the yield was 11.9%. The very high cellular zinc in this case may indicate an error in the cell count.
- With the exception of one women (# 10) the number of platelets was good, 273 to 1071 x 10^3 / µL. The number of platelets isolated from plasma from # 10 was 57 x 10^3/UL. The platelet zinc was at the lower limit of normal and this subject who also had low granulocyte and low lymphocyte zinc.
- The variability in cellular zinc is similar to that reviewed by others (45, 46). Little data is available on platelet zinc other than that of Wang et al (43). Currently the precision of the method is being determined from cell isolation and zinc analysis from blood collected on 10 separate days from a single individual .
- Hair zinc varies with nutritional status. The low level in subject # 13 together with the cellular zinc indicates poor nutritional status.

The significance of these data will be determined after more data are collected and compared to results of zinc kinetics.

2. Beta-hydroxybutyrate is being measured because findings in rats (47) indicate severe zinc deficiency increases plasma beta-hydroxybutyrate.

- The values ranged from 0 - 1.6 mg/dL Many were at or below the detection level (0.21 mg/dL).
- All levels were within the reference range (0 - 4.39 µg/dL).
- No significant difference was detected in the value obtained at baseline, and at various periods of storage at -20°C or -70°C up to 123 days.
- Quality control for this assay was a normal control serum. The mean value obtained was 6.54 mg/dL (SD 0.277), N=12; expected value 6.0 mg/dL. The mean value obtained on an elevated serum - expected value 50.0 mg/dL - was 49.6 (SD 2.95), N=10.
- Sufficient data has been obtained to determine that plasma may be stored at -70° C and the assay performed with accumulated batches of specimens.

3. Zinc kinetics were measured in staff and subjects by isotope ratio inductively coupled plasma-mass spectrometry (ICP-MS) analysis of intravenously injected ^{67}Zn relative to stable zinc isotopes in blood and urine. Zinc status of staff and subjects was determined from kinetic (k, zinc pool sizes, etc.) and plasma Zn. Relationships between zinc kinetics and indices of body composition were determined, and renal handling of zinc assessed.

Data from these studies have been presented as posters (see appendix) at two 1996 scientific meetings, the Central Society for Clinical Research and the American College of Nutrition.

To accomplish these measurements collection of blood and urine samples was modified from that described above. Following collection of the baseline blood sample and intravenous administration of 2 mg of ^{67}Zn, blood was taken after 5, 15, 30, 40, 50, 60, 90 minutes, 2, 6, 12 hours, 1, 2, 3, 5, 7, and 9 days. Urine samples were collected after 1, 2, 3, 4, 5, 6, 7, 8 and 9 days.

Extraction and analysis of the samples was done as described above and reported (1, 18). The following isotope ratios were determined: ^{67}Zn:^{64}Zn, ^{67}Zn:^{66}Zn, ^{67}Zn:^{68}Zn, ^{67}Zn:^{70}Zn.

The above data were used to determine the following:

- The normalized zinc isotope ratio (NIR) was determined by subtraction of the natural baseline zinc isotope ratio from the measured isotope ratio, divided by the natural zinc isotope ratio.

- The exchangeable zinc pool (EZP) as a norm was calculated using the open 'mammillary' model of Landaw et al (48) from the obtained coefficients of the polyexponential function fitted to the 5 minute to 9 day plasma data.

- The 5 minute to 24 hour EZP was calculated using the closed 'mammillary' model from the extrapolation of logarithm of NIR of 3 to 9 day plasma and urine; from the NIR of the 24 hour plasma; and from the 24 and 48 hour spot urines.

- Relationships between EZP and indices of body composition were assessed.

- The urinary zinc elimination constant was determined and compared with the plasma zinc concentration.

Anthropometric characteristics are shown in Table 2.

Table 2. Anthropometric Characteristics

Initial	Weight (kg)	Height (m)	BMI (kg/m^2)	Lean Weight (kg)	Age (years)
Males					
1	77.2	1.802	23.8	64.8	44
2	93.2	1.727	31.3	60.1	63
3	70.2	1.692	24.5	56.3	32
4	78.4	1.725	26.4	61.2	42
5	83.5	1.842	24.6	67.5	24
Females					
6	71.9	1.758	23.3	51.8	24
7	50.9	1.640	18.9		26
8	65.9	1.725	22.2	48.5	26
9	59.9	1.740	19.8		31
10	56.8	1.680	20.1	42.5	24
11	54.0	1.620	20.6	41.9	24
12	61.8	1.680	21.9	45.3	28
13	51.1	1.600	20.0	39.2	39
14	54.2	1.684	19.1	42.0	28
15	47.0	1.535	20.0	34.0	29
16	58.6	1.660	21.3	44.1	22

The calculated disappearance constant k from the ICP-MS isotope ratio analyses and the plasma Zn levels from AA-analyses are presented in Table 3. Table 4 shows serum ferritin concentration, the plasma Zn binding capacity, the urinary Zn and creatinine excretion and the rapidly exchangeable Zn pool of each of the subjects.

Two women, had the slightly elevated 30 and 60 minute plasma Zn disappearance constant k (Table 4). Their plasma zinc (Table 4) and serum ferritin (Table 5) concentrations were within the normal range (>700 ng/mL and >20 ng/mL, respectively). The plasma zinc binding capacity (Table 4) of our women subjects was 4600 to 6200 ng/mL. Argemi et al (49) reported (mean±SD) 5497±982 ng/mL in 14 normal women. The plasma zinc binding capacity of our subjects was within ± 1 SD of Argemi's data.

Table 3. Disappearance constant k and plasma Zn of the subjects

Initial	Disappearance constant k (min^{-1})	Diagnosis of k	Plasma Zn (ng/mL)	Diagnosis of plasma Zn
Males				
1	0.0188	Normal	880	Normal
2	0.0200	Normal	798	Normal
3	0.0188	Normal	959	Normal
4	0.0236	Elevated	979	Normal
5	0.0179	Normal	1023	Normal

Table 3. Disappearance constant k and plasma Zn of the subjects (continued)

Initial	Disappearance constant k (min^{-1})	Diagnosis of k	Plasma Zn (ng/mL)	Diagnosis of plasma Zn
Females				
6	0.0182	Normal	796	Normal
7	0.0190	Normal	846	Normal
8	0.0226	Elevated	939	Normal
9	0.0188	Normal	929	Normal
10	0.0188	Normal	811	Normal
11	0.0172	Normal	745	Normal
12	0.0205	Borderline	781	Normal
13	0.0200	Normal	700	Borderline
14	0.0235	Elevated	876	Normal
15	0.0178	Normal	781	Normal
16			907	Normal

Table 4. Ferritin; Zn Binding Capacity; 24-h Urine Zn; & 24-h Urine Creatinine

Initial	Serum ferritin (ng/mL)	Plasma Zn binding capacity (ng/mL)	24-h Urinary Zn excretion (μg/d)	24-h Urinary creatinine excretion (g/d)	Rapidly exchangeable Zn pool (mg)
Males					
1	47	5738	729	2.08	202
2		5956	572	2.00	251
3	351	5692	854	1.69	213
4		6214	831	2.01	244
5		6295	818	1.98	194
Females					
6	7	6232	455	1.69	182
7	35	5732	485	0.98	122
8	29	6182	549	1.38	176
9	78	4729	508	1.24	160
10	7	5712	358	1.00	129
11	50	5303	248	1.17	106
12	56	4641	385	1.22	158
13	11	5272	196	1.03	125
14	31	4968	312	1.00	140
15	45	5913	191	0.74	121
16	17	5681	283	1.27	132

Zinc pool sizes and turnover rates (Table 5) were measured based on the closed mammillary model. The general formulation of the mammillary model was based on Landaw et al (48).

Table 5. Zn pool sizes (mg) and the plasma Zn turnover rate (mg/d)

Initial	Rapidly exchangeable Zn pool (= Q1 + Q2 + Q3)	Q1 (Plasma Zn pool)	Q2 (Mainly liver pool)	Q3 (Muscle & other tissue pool)	Plasma Zn turnover rate
Males					
1	202.18	4.29	19.73	178.16	420.72
2	250.78	4.94	29.83	216.02	550.47
3	213.26	4.86	39.81	168.59	513.02
4	243.75	4.35	35.35	204.04	520.70
5	194.48	3.98	28.86	161.63	562.50
Females					
6	181.80	2.84	28.90	150.06	449.68
7	121.90	3.18	14.43	104.29	420.56
8	175.70	3.45	11.12	161.13	419.25
9	160.40	3.50	19.26	137.63	431.45
10	128.89	2.75	13.47	112.67	321.88
11	105.78	2.43	11.33	92.01	283.79
12	157.75	3.27	13.63	140.85	358.98
13	125.01	2.46	10.84	111.72	291.92
14	133.44	2.46	20.84	110.14	251.52
15	134.20	2.20	12.91	106.18	307.1
16	131.89	3.03	11.19	117.67	330.33

Exponential coefficients from the truncated model described in previously are shown in Table 6.

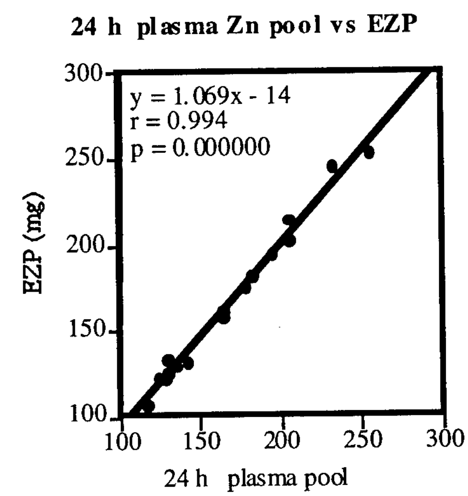
Table 6. Exponential coefficients (day^{-1}) from the truncated model

Initial	g_1	g_2
Males		
1	106.8	5.260
2	118.7	4.823
3	112.5	3.147
4	126.4	3.747
5	149.9	5.119
Females		
6	165.6	4.086
7	143.7	7.511
8	129.4	9.403
9	132.3	5.763
10	126.8	5.964
11	127.8	6.013
12	117.1	6.913
13	127.6	6.974
14	107.4	3.23
15	123.1	6.476
16	123.1	6.476

- Validity of the 24 hour Plasma Zinc Pool Method for the Estimation of EZP

The 24 hour plasma zinc pool (based on a single plasma collected 24 hours after injection of ^{67}Zn) and the rapidly exchangeable zinc pool (EZP), calculated from the truncated model based on the 5 minute to 24 hour plasma data, were highly correlated (Figure 1). Thus the EZP can be determined by assay of a single plasma sample.

Figure 1



- Body Composition and EZP

The association between body weight and EZP is shown in Figure 2; figure 3 shows the association in females. The association was highly significant with negligible intercepts (r = 0.925, p = 0.0000 for all subjects; r = 0.891, p = 0.0002 for females). This finding indicates the amount of exchangeable zinc per unit of weight is constant under normal conditions. For all subjects the mean, SD and CV of EZP were 166 mg, 45 mg and 27 %, and the mean, SD and CV of the EZP per kilogram weight were 2.54 mg, 0.28 mg and 11 %.

Figure 2

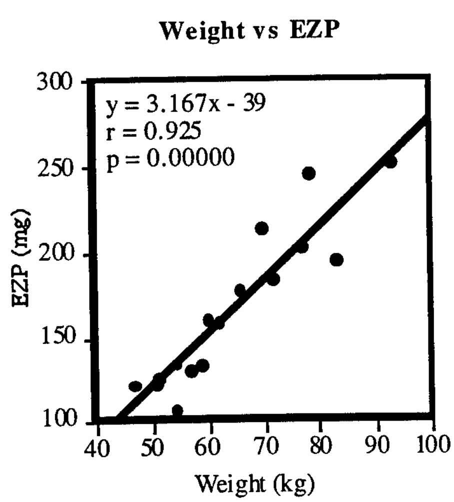
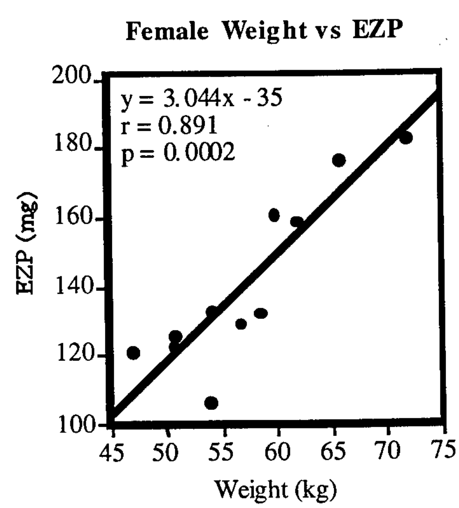


Figure 3



Lean weight was determined by the Bioelectrical Impedance. Lean weight was strongly associated with the EZP (Figure 4, all subjects; Figure 5, females).

Figure 4

Lean Weight vs EZP

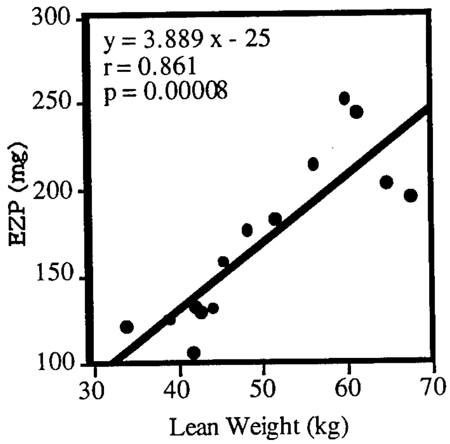
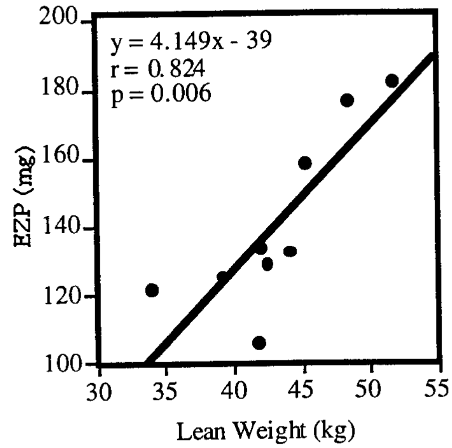


Figure 5

Female Lean Weight vs EZP



Fat mass was associated with EZP (Figure 6, all subjects; Figure 7, females). However the regression lines for males and females were different.

Figure 6

Fat mass vs EZP

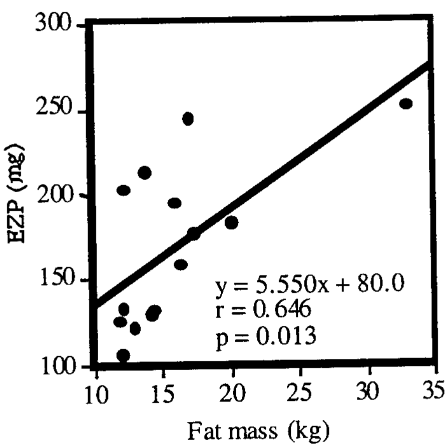
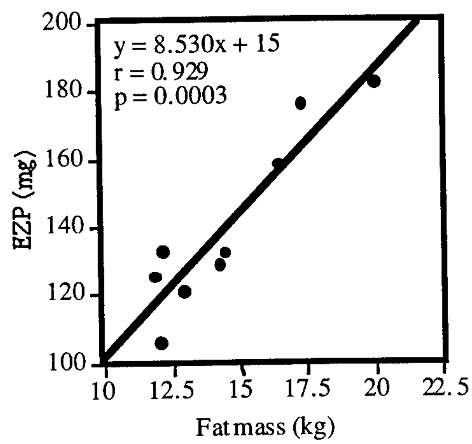


Figure 7

Female Fat mass vs EZP



The 24 hour urine creatinine excretion and the EZP were highly correlated (Figure 8, all subjects; Figure 9, females).

Figure 8

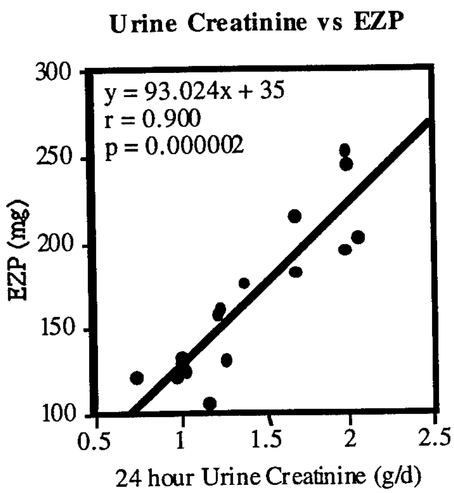
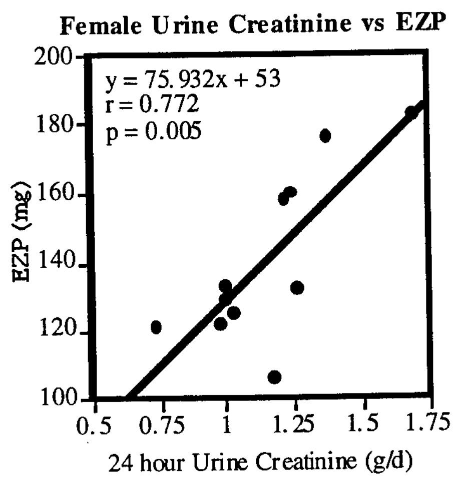


Figure 9



The negligible intercept of the regression line for the skeletal muscle mass derived from the 24 hour urine creatinine excretion (50) suggests that most of the EZP is in skeletal muscle (Figure 10, all; Figure 11, females).

Figure 10

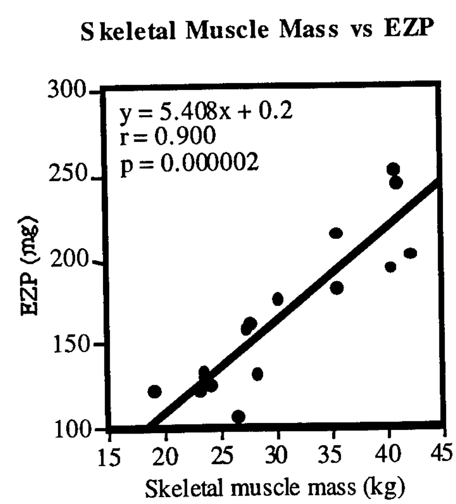
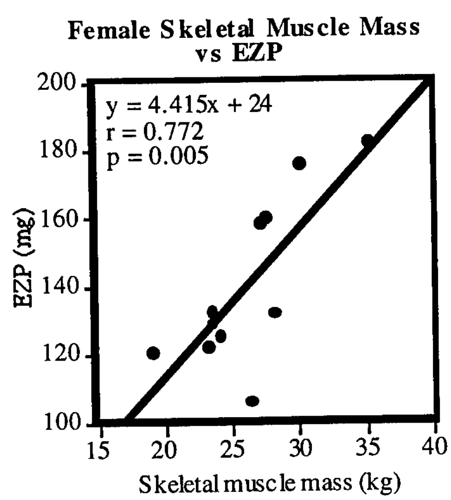


Figure 11



In contrast, non-skeletal-muscle fat free mass (lean body weight minus skeletal muscle mass) and EZP did not show a strong correlation (Figure 12, all subjects; Figure 13, females). This finding suggests that the non-skeletal-muscle fat free mass (NSMFFM), including the visceral mass, is not be an important contributor to the EZP.

Figure 12

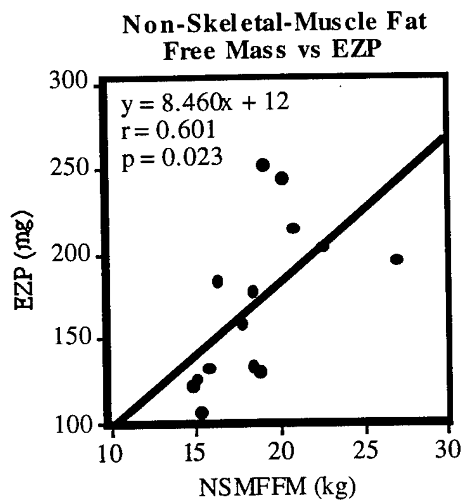
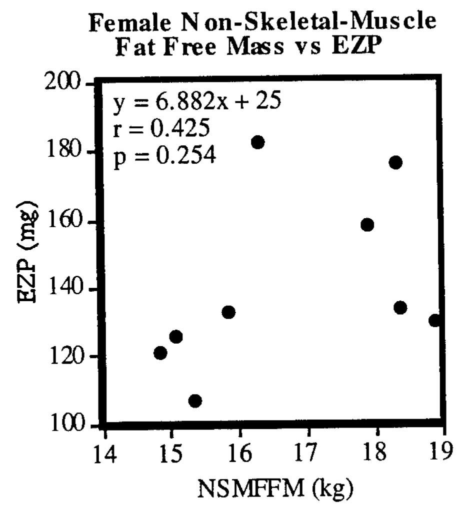


Figure 13



- EZP and Plasma Zinc Turnover Rate

Figure. 14 shows the association between lean body weight and the plasma zinc turnover rate for all subjects. The plasma zinc turnover rate is determined by the initial slope and the extrapolated intercept of the disappearance constant, while the EZP is determined by the 24 hour plasma zinc data.

Figure. 14

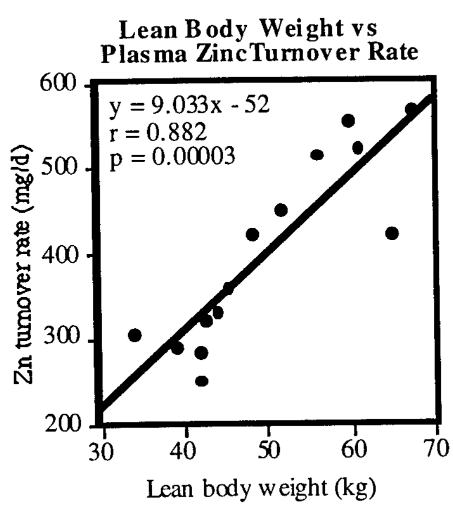
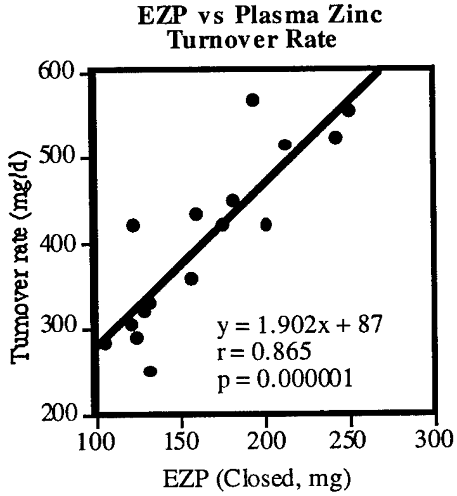


Figure 15 shows the association between the EZP and the plasma zinc turnover rate. Because the plasma zinc turnover rate is about 300 to 600 mg/d and far above the zinc excretion rate of about 2 mg/d, it is virtually identical to the zinc uptake rate by tissues. The EZP reflects the metabolically active tissue mass. Therefore, it appears we observed the same metabolically active mass both initially (turnover rate) and 24 hours later (EZP) through behavior of the tracer.

Figure 15



- Plasma Zinc as a Determinant of Urinary Zinc Excretion

Figure 16 shows the association between plasma zinc concentration and the 24 hour urinary zinc excretion. Although the association is clear, the pool sizes of the subjects are different. The urinary zinc elimination constant was obtained by dividing 24 hour urinary Zn excretion by the plasma Zn compartment size (= Q1).

Figure 16

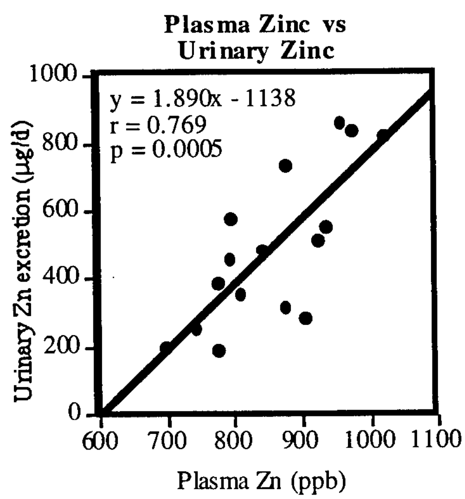


Figure 17 shows the association between the urinary zinc elimination constant and the plasma zinc concentration. The extrapolated X intercept is 0.433 µg/mL when the elimination constant is 0 day $^{-1}$. This finding shows that the urinary elimination constant of zinc is proportional to the plasma zinc concentration minus 0.433 µg/mL (threshold). This is consistent with non-linear homeostatic regulation of urinary zinc excretion. When plasma zinc is low, the urinary zinc elimination constant is decreased and urinary zinc excretion is more decreased than predicted by linear kinetics, and zinc is spared.

Figure 17

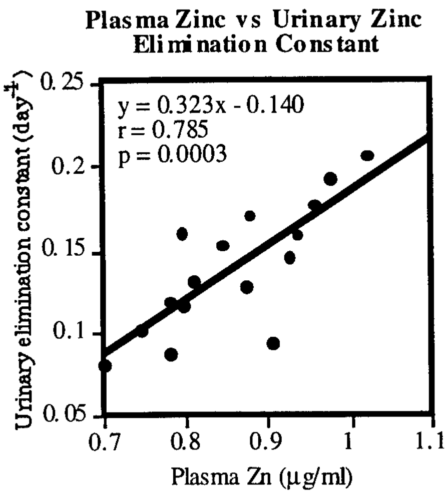
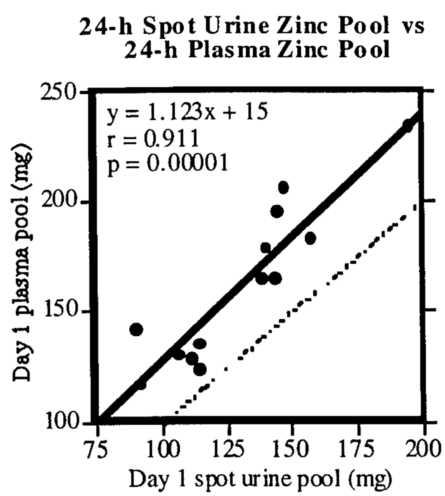


Figure 18 shows the association between the exchangeable zinc pools derived from the tracer in plasma and urine 24 hours after administration. The pool derived from tracer in urine is smaller than that derived from tracer in plasma. Miller et al (17) described a similar phenomenon, based on the 3 - 9 day extrapolation of the percent tracer enrichment of urine and plasma, and suggested the cause was a time lag between glomerular filtration and micturition. We suggest an alternative explanation. About 20 % of zinc in plasma is tightly bound to an α2-macroglobulin and turns over slowly. Little injected tracer enters the α2-macroglobulin pool within 24 hours and very little zinc in the α2-macroglobulin pool appears in the urine during the 24 hours after tracer administration. Thus 24 hours after tracer administration the normalized zinc isotope ratio in urine is about 20 % higher than in plasma.

Figure 18



Plans Next Quarter

Aggressively identify potential candidates. This will require screening of more than 40 respondents. From these we will select and randomize at least 15 subjects according to iron and zinc status and will enter at least 10 subjects into the treatment trial.

(7) Conclusions

- It is considerably more difficult to identify study subjects than planned. The primary reasons are stringent selection criteria. Such criteria are needed so that the final product is interpretable.
- Technically the yield and purity of leukocytes and platelets is good by the isolation technique.
- The zinc concentration in cells is consistent with published reports.
- The ICP-MS methodology is highly satisfactory. Measurements of isotope ratios $^{67/68}Zn$, $^{67/64}Zn$, and $^{67/66}Zn$ are accomplished with a Coefficient of Variation of < 1 %.
- The R^2 of the "truncated" 24 hour model fitted to the isotope ratio data (disappearance) after intravenous administration of ^{67}Zn was 0.993.
- The "truncated" 24 hour model is concordant with the triexponential model applied to 9 days of observation (see the March and June reports).
- The finding reported in March, that the 24 hour plasma zinc pool size determined by analysis of plasma obtained 24 hours after administration of tracer is equivalent to the rapidly exchangeable zinc pool size determined by the "truncated" model, has been confirmed. We now have data from 16 subjects.
- The derived exchangeable zinc pool size at 24 hours is systematically about 20% larger than the exchangeable zinc pool derived from the open mammillary model after 9 days. The open model accounts for losses of tracer and quasi-equilibrium. Thus the 24 hour is a useful index of zinc status.
- The 24 hour EZP is highly correlated with body weight (r= 0.925), lean body weight (r= 0.861), and creatinine excretion (r= 0.900).
- The urinary zinc elimination constant is correlated well with plasma zinc (r= 0.785).
- The 24 hour spot urine zinc pool is about 20% less than the 24 hour spot plasma zinc pool (r= 0.911).
- We are unable to comment on behavioral findings because this is a double-blind study.

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Principal Investigator: Sandstead, Harold H.
Contract No. DAMD17-95-C-5112

QUARTERLY REPORT

1. Contract No.:	DAMD17-95-C-5112	2. Report Date:	December 22, 1995
3. Reporting Period from:	September 22, 1995	to	December 22, 1995
4. PI:	Harold H. Sandstead	5. Telephone No.	(409) 772-4661

6. Institution: The University of Texas Medical Branch

7. Project Title: Repletion of Zinc and Iron deficiencies improves cognition of premenopausal women.

8. Current Staff, with percent effort of each on project:

Harold H. Sandstead	15 %	Nancy W. Alcock	10 %
VM Sadagopa Ramanujam	25 %	Hari H. Dayal	10 %
Norman G. Egger	100 %	Jackie Callies	60 %
Katsuhiko Yokoi	25 %		

9. Contract expenditures to date (as applicable):

	This Otr/Cumulative		This Otr/Cumulative
Personnel:	14,778.61 / as of 12/28/95	Travel:	NA / as of 12/28/95
Fringe Benefits:	2652.46 / as of 12/28/95	Equipment:	NA / as of 12/28/95
Supplies:	15,575.04/ as of 12/28/95	Other:	NA / as of 12/28/95

	This Otr/Cumulative
Subtotal:	33,006.11 / as of 12/28/95
Indirect Costs:	9222.64/ as of 12/28/95
Fee:	NA / as of 12/28/95
TOTAL:	**42,228.75/ as of 12/28/95**

10. Comments on administrative and logistical matters.

We experienced delay in ^{67}Zn from Oakridge National Laboratory because of the requirement we accept bids.

11. Use additional page(s), as necessary, to describe scientific progress for the quarter in terms of the tasks or objectives listed in the statement of work for this contract. Explain deviations where this isn't possible. Include data where possible.

a) The research group met weekly to review progress. There was some delay because the physician (Norman Egger, M.D.) was finishing work with another investigator.

b) The decrease in the budget required a decrease in personnel. Therefore, a post graduate physician was hired instead of a physician's assistant (PA). He will carry out duties proposed for the PA, in addition to assisting with the ICP-MS assays, measuring respiratory quotients, involuntary muscle strength, taste thresholds, and carry out other duties as assigned. Salary saved is being used to support a visiting scientist for 6 months. He is an expert in ICP-MS, having worked with us on research that showed low ferritin is associated with a rapid plasma disappearance of ^{67}Zn. In addition to assisting with the ICP-MS work, he is setting up assays to measure calcium channels and protein kinase-C. These assays are part of our effort to maximize information obtained from this project.

c) We are purchasing initial equipment and supplies. Items specified have been or are in the process of being ordered.

d) While waiting for the ^{67}Zn order to arrive we prepared ^{67}Zn we have in stock for study of 10 subjects.

e) The project was advertised through a press release, an interview with a local radio commentator, and notice in the Campus Weekly. Posters will be put up after January 1. Sixteen respondents inquired to date. Nine satisfy the initial criteria.

f) The forms **(attached)** have been revised and extended. New forms have been added where necessary. Professor Dayal was particularly helpful. .

g) The method for ICP-MS measurement of the $^{67}Zn:^{68}Zn$ ratio has been validated. Professor Ramanujam's report is attached. ***(Addendum I)***

h) The methods for isolation of white blood cells and platelets, and serum ß-hydroxy butyrate have been validated. The assay for transferrin receptors will be set up soon. Professor Alcock's report is attached. ***(Addendum II)***

i) Other efforts to extend the data collected from this project include: measurement of serum and urine indicators of bone metabolism (by a colleague in the Department of Orthopedics), steroid receptors on white blood cells (by a colleague in the Department of Human Biological Chemistry and Genetics), serum amino-acid concentrations (by a colleague in the Department of Human Biological Chemistry and Genetics), amino-acid utilization for synthesis of rapidly turning over proteins (by a colleague at the USDA, ARS Children's HNRC, Houston, TX), serum neuro-hormones (by a colleague at the USDA, ARS HNRC, Beltsville, MD), serum and RBC folate, vitamin B_{12}, pyridoxin and homocysteine (by a colleague at the University of Alabama, Birmingham, AL).

j) We identified colleagues who prepared applications to the 1996 US Army Research Program on Women's Health. These projects would utilize the subjects from our current project. The two applications from UTMB focus on effects of mild zinc and iron deficiencies on aspects of cell mediated immunity (Department of Microbiology); and the effects of mild zinc and iron deficiencies on brain function as measured by Magnetic Resonance Imaging (Department of Radiology). The other application is from the Department of Medicine at Wayne State University Medical School, Detroit, MI. It examines aspects of immunity that are not covered by the project from UTMB.

k) We changed the times of blood drawing so that we can measure the rapidly turning over pool size as well as the ^{67}Zn disappearance during the second phase of the curve.

l) We submitted an application to the USDA extra-mural grants program and will submit it to the NIH. The project will measure effects of zinc and iron treatments on plasma ^{67}Zn disappearance and pool sizes at follow-up. Subjects in the last two years of this project will be studied.

12. Use additional page(s) to present a brief statement of plans or milestones for the next quarter.

a) Measure relation of ^{67}Zn disappearance and pool size to white blood cell zinc in five of the Co-investigators while we are screening subjects. We will use ^{67}Zn we have in stock for this activity.

b) Enter ten subjects into the project.

c) While subjects are being screened we will measure the Zn disappearance rate, rapidly exchangeable pool (2-compartment model) and white blood cell Zn in five of us. This will further confirm our methods. We will also measure the above indices in subjects as they enter the study.

ADDENDUM I

1. Purchase of Items Necessary for Zinc Kinetics

a) The quantity of ^{67}Zn necessary for 100 subjects (over three years) was is 250 mg ^{67}Zn oxide. An order has been sent to Oak Ridge National Laboratories (ORNL). The purchase is delayed because of the requirement that the order be sent out for bids.

b) Inductively Coupled Plasma-Mass Spectrometry Items:

3 Quartz torches for PlasmaQuad-1 and 3 Quartz elbow have been purchased from the Precision Glass Blowing company, Englewood, Colorado.

The following have been ordered from Fisons, Beverly, Mass.: Mini-skimmer nickel kit (box of 3), Nickel sampling cone kit (box of 3), polythene tubing, Tygon tubing, Swagelok nylon elbow, Swagelok nylon coupling, and PTFE ferrules.

c) Laboratory Glassware and other Supplies:

Pyrex glass tubes with Teflon coated screw caps for zinc extractions have been purchased from Fisher Scientific.

Other laboratory supplies such as: polypropylene tubes (50 mL and 14 mL volumes) for digestion, Li-Heparin LH/25 Monovette syringes for blood collection, and Pasteur pipettes are available in small quantities for immediate use, and more will be purchased when needed.

d) Chemicals, Acids, Solvents, and other Reagents:

Small quantities of hydrogen peroxide (30%, for digestion), hydrochloric and nitric acids, ammonium hydroxide, 2,6-dinitro phenol (pH indicator), diethylammonium diethyldithiocarbamate (Zn chelating agent), carbon tetrachloride and ethanol solvents, Zn and yttrium standards (for ICP-MS analyses), and (glassware cleaning solution) are in stock. More will be purchased when needed.

2. Zinc Kinetics Methodology

Even though our laboratory has been using this methodology for several years, we felt it essential to test each steps involved. For these experiments, our published procedures were used.

a) Lyophilization and digestion of plasma sample to white ash. The procedure requires at least four days, two days for lyophilization and two days for digestion. Digestion was done at 80°C in an heating oven. Digestion of each 2 mL sample requires about 10 mL of 30% hydrogen peroxide.

b) The isolation of Zn from the ash. The ash was dissolved in dilute nitric acid (1.2 M). The pH was adjusted using 2,6-dinitrophenol. The Zn was extracted with diethylammonium diethyldithiocarbamate (0.25%) in carbon tetrachloride for (30 sec mixing for each tube) and the layers separated. Then the Zn was extracted back into the dilute nitric acid (4 min mixing for each tube). Yttrium internal standard (100 µL of 5 ug/mL in 10 mL volume) was added to each tube for ICP-MS measurements. The time involved was 8 hours for 10 tubes of digested ash samples.

c) The Zn recovery was established by extracting known amounts (2.5, 5, 7.5 and 10.0 ug) of Zn standard and performing "fully quantitative" analysis mass spectral acquisition algorithm of the ICP-MS instrument and found to be each greater than 99%.

d) The ICP-MS isotope ratio measurements. Measurements were done using both Zn standards (100, 250, and 500 ug/L samples) and isolated Zn from plasma. The mass spectral acquisitions were performed using the previously established peak jump and scan acquisition instrumental parameters. As we observed previously, for all the four chosen Zn isotope ratios 67/64, 67/66, 67/68, and 67/70, the peak jump isotope ratio mass spectral acquisition mode gave better precision and accuracy (CV< 1%) compared to values from scan acquisition mode (CV > 2%) for similar mass spectral parameters.

   From several ICP-MS isotope ratio measurements, we found the best mass spectral parameters for this study include peak jump mode with 100 peak jumps, 200 scan sweeps, 160 μsec dwell time, and 10 consecutive measurements per sample tube.

   From these experiments it has been estimated that it will take about 7-8 days to determine the Zn disappearance rate constants (10 data points containing duplicate measurements) on each subject.

3. Enriched ^{67}Zn Solution

The solution was prepared from stock on hand. The ^{67}Zn (94 atom %) oxide (29.76 mg) was dissolved in a few drops of Ultrapure hydrochloric acid and the solution heated on a hot plate almost to dryness. The ^{67}Zn chloride was dissolved in 6 mL saline (0.9% sodium chloride solution). Each 0.5 mL corresponds to 2 mg equivalent of ^{67}Zn. The solution was filtered thru Millipore filter (0.2 μm) and 0.5 mL aliquots were added to each 10 mL sterile solution vial and sealed (by the UTMB pharmacy). One vial is being tested for pyrogenicity by Scientific Associates Inc., St. Louis, MO. Another is being tested for sterility by the UTMB Department of Clinical Microbiology and Immunology.

ADDENDUM II

Preparation of lymphocytes, granulocytes and platelets.

The isolation of white cells and platelets from whole blood has been assessed. The results shown in the attached table, indicate that the granulocyte fraction is relatively pure. More than 94% of the cells in each of preparations were granulocytes. Red cell and platelet contamination was almost entirely absent.

The lymphocyte fraction was variable in the 3 preparations. This may be due to the incomplete separation of the 2 ficoll-hypaque densities, which improved with practice of their "layering". As a result, variable contamination with monocytes was present.

The platelet fraction had negligible red cell contamination and some monocytes were present.

The procedure for zinc determination by graphite furnace analysis was optimized. Samples of white blood cells will be digested with hydrogen peroxide for analysis.

QUARTERLY REPORT

1. Contract No.: DAMD17-95-C-5112 2. Report Date: March 22, 1996

3. Reporting Period from: December 23, 1995 to March 22, 1996

4. PI: Harold H. Sandstead 5. Telephone No. (409) 772-4661

6. Institution: The University of Texas Medical Branch

7. Project Title: Repletion of Zinc and Iron deficiencies improves cognition of premenopausal women.

8. Current Staff, with percent effort of each on project:

Harold H. Sandstead	15 %	Nancy W. Alcock	10 %
VM Sadagopa Ramanujam	25 %	Hari H. Dayal	10 %
Norman G. Egger	90 %	Jackie Callies	60 %
Katsuhiko Yokoi	25 %		

9. Contract expenditures to date (as applicable):

	This Otr/Cumulative		This Otr/Cumulative
Personnel:	46,393.35 / 61,171.96	Travel:	NA / NA
Fringe Benefits:	8,779.31 / 11,431.77	Equipment:	2443.00 / 2443.00
Supplies:	12,204.34/27,779.38	Other:	NA / NA

	This Otr/Cumulative
Subtotal:	69,820.03 / 102,826.14
Indirect Costs:	29,562.89/ 38,785.53
Fee:	NA / NA
TOTAL:	**99,382.92/ 141,611.67**

10. Comments on administrative and logistical matters.

    Treatment delivery was delayed by the supplier. We have been assured the treatments will be delivered in early April.

11. Use additional page(s), as necessary, to describe scientific progress for the quarter in terms of the tasks or objectives listed in the statement of work for this contract. Explain deviations where this isn't possible. Include data where possible.

    a) The research group met weekly.

    b) All equipment and supplies were received.

    c) Fifty-five women were interviewed by telephone; twenty one were screened by detailed medical and dietary histories and laboratory measurements (phase 1); nine met the criteria for measurement of Zn kinetics (phase 2).

    d) The Food Frequency Questionnaires were revised and extended **(attached).** We will administer the questionnaire at Screening, Baseline, Cross over, and End of study.

    e) Co-Investigator Penland provided forms for collection of subjective symptoms related to the menstrual cycle **(attached).** These forms are similar to those used by Penland in studies of women at the USDA, ARS Human Nutrition Research Center in Grand Forks, ND.

    f) A revised subject consent form that identifies add-on measurements and gives more details of the project was approved by the UTMB IRB **(attached).** Subjects will thus be better informed. The form was otherwise unchanged.

    g) The CRC dietitian prepared a diet that provides about 3.9 mg Zn daily **(attached)**. Subjects will be fed the diet during their admissions to the CRC.

    h) A modem connection was set up for direct transfer of neuropsychological data from the UTMB Clinical Research Center to Co-Investigator Penland at the USDA ARS Human Nutrition Research Center in Grand Forks, ND.

    i) The method for zinc kinetics was validated **(Addendum I)**.

    j) Other laboratory methods were validated. **(Addendum II)**.

    k) The randomization method was refined. **(Addendum III)**.

    l) An application was submitted to the NIH to measure changes in Zn kinetics after the treatments. Subjects would be from the last two years of this project.

12. Use additional page(s) to present a brief statement of plans or milestones for the next quarter.

    a) Screen 40 potential subjects (phase 1) and measure Zn Kinetics in ten experimental subjects (phase 2), begin treatments (phase 3).

    b) Measure Zn kinetics in three additional normal adult men.

    c) Accumulate additional data showing that simple principles of isotope dilution allow the use of single plasma and urine samples obtained 24 hours after isotope infusion for calculation of Zn exchangeable pool size.

    d) Accumulate additional data showing the validity of the truncated model.

ADDENDUM I **(prepared by K Yokoi, VMS Ramanujam, and NG Egger)**

Refinement of the ICP-MS Methodology and Measurements of Zinc Kinetics

Zinc kinetics were measured in two men (KY and HHS). The purpose was the following:

- Refine the ICP-MS methodology.

- Compare ICP-MS results (Zn isotope ratios) when unextracted plasma digestates are used compared to extracted plasma digestates.

- Compare the rate constants and pool sizes from both types of samples.

Subjects

Two healthy men.

Enriched Zinc-67 Stable Isotope Solution

The solution was prepared using ^{67}Zn (94 atoms %) oxide. The ^{67}Zn oxide (29.76 mg) was dissolved in a few drops of Ultrapure hydrochloric acid and heated almost to dryness. The resulting ^{67}Zn chloride was dissolved in 6 mL saline (0.9 % sodium chloride solution). Each 0.5 mL corresponded to 2 mg equivalent of ^{67}Zn. This solution was then sterilized and bottled by the UTMB Pharmacy. After filtering thru a Millipore 0.2 µm filter, 0.5 mL aliquots were added to each 9.5 mL sterile saline in a sealed vial. The solution was then demonstrated pyrogen free (Scientific Associates Inc., St. Louis, MO) and sterile (UTMB Department of Clinical Microbiology and Immunology).

Blood sampling and Plasma Isolation

Catheters were inserted into each antecubal vein. A slow running infusion of 0.5 N saline was attached to the right sided catheter. The left sided catheter was locked with heparin. After 30 minutes a baseline blood was taken from the left catheter. Two milligrams of ^{67}Zn was then injected iv., in 30 mL of 0.5 N saline over 3 minutes into the saline infusion stream. Saline was then infused rapidly for 30 seconds to flush the isotope into the blood stream. Blood was then taken from the left catheter at the following times (minutes after isotope administration): 5, 15, 30, 40, 50, 60, 90, 120, 360, 720, 1440. Thereafter blood was collected after 3, 5, 7, 9, and 22 days. Total blood drawn was about 275 mL. Blood collected using Sarstedt syringe was immediately put on ice until centrifugation at 2000 rpm for 20 min. The plasma recovery was about 40% of the whole blood volume.

In duplicate 1.5 -3 mL plasma was transferred to falcon tubes and frozen at -70^{0}C until preparation for extraction of Zn. Urine was collected for one hour, at about the same time, on the morning of each blood sampling day for a spot sample.

Digestion of Plasma Samples

Plasma samples were lyophilized (24 hours) and digested to a white ash with pure 30 % hydrogen peroxide (at 80°C in an heating oven for 3 days) using our previously published procedure. Digestion of each plasma sample (2 mL) required approximately 10 mL of hydrogen peroxide.

Extraction and Isolation of Zinc

The isolation of zinc by extraction procedure involves the following steps:

- Dissolution of the ash in dilute nitric acid (1.2 M), pH adjustment (using 2,6-dinitrophenol)

- Extraction using diethylammonium diethyldithiocarbamate chelating agent (0.25 %) in carbon tetrachloride (40 sec mixing for each tube)

- Each extraction of zinc in dilute nitric acid (4 min mixing for each tube)

- Addition of yttrium internal standard (100 µL of 5 µg/mL for each mL sample volume)

The ICP-MS isotope ratio measurements

We previously showed the best mass spectral acquisition parameters for the isotope ratio measurements are pulse counting with the detectors in the peak jump mode, 100 peak jumps with 200 scan sweeps, 160 sec dwell time, 2048 channels, and 10 consecutive measurements per sample tube. As we observed previously, the peak jump isotope ratio mass spectral acquisition mode gave better precision and accuracy (Coefficient of Variation, C.V., less than 1%) compared to values obtained using the scan acquisition mode (C.V. greater than 2%) for similar mass spectral parameters, for the four Zn isotope ratios, 67/64, 67/66, 67/68, and 67/70.

Results and Discussion

Plasma Zn isotope ratios were obtained from two men (KY and HHS) after intravenous administration of 2 mg (94 % purity) ^{67}Zn.

To avoid interferences from $^{32}S_2$ (32 x 2 = 64 atomic mass units) and/or $^{32}S^{16}O_2$ (32 + 16 x 2 = 64 atomic mass units), isotope ratios $^{67/66}Zn$ and $^{67/68}Zn$ were normalized using the natural zinc isotope ratios (0.146953 and 0.21867). All kinetics analyses were done using the natural logarithm of the normalized isotope ratios.

The $^{67/66}Zn$ and $^{67/68}Zn$ isotope ratios were almost identical between extracted and unextracted samples at least within 24 hours when the normalized ratio was at least 20 % greater than the baseline (Figure 1).

The disappearance constants k are shown in Table 1. The disappearance constants calculated from extracted and unextracted samples were similar.

Data from 5 minutes to 9 or 26 days were analyzed by the nonlinear regression of Systat Software using a tri-exponential function model (Table 2; Figure 2). The model is as follows:

Log (Normalized ratio) = Log [$K_1 \cdot$ Exp (- $g_1 \cdot$ Day) + $K_2 \cdot$ Exp (- $g_2 \cdot$ Day) + $K_3 \cdot$ Exp (- $g_3 \cdot$ Day)]

K_1:Proportional coefficient for the second term	g_1: Corresponding exponential coefficient
K_2:Proportional coefficient for the second term	g_2: Corresponding exponential coefficient
K_3:Proportional coefficient for the third term	g_3: Corresponding exponential coefficient

Pool sizes and turnover rates were calculated based on our simplified interpretation (Appendix).

The derived pool sizes and turnover rates are comparable between subjects (Table 3),

g_3 was about 0.1. Within 24 hours the change of the third term was less than 10 %. Therefore, the truncated model was applied using the 24 hour data.

$$\text{Log (Normalized ratio)} = \text{Log}\,[K_1 \cdot \text{Exp}(-g_1 \cdot \text{Day}) + K_2 \cdot \text{Exp}(-g_2 \cdot \text{Day}) + K_3]$$

The results are shown in Tables 4, 5 and 6 and Figure 3. With the exception of the second term exponential coefficient (g_2) and the corresponding turnover rate, all values agreed well between the tri-exponential model and the truncated model (except tri-exponential model of the unextracted samples of KY).

The parameters obtained from the extracted and unextracted plasma samples were compared using the truncated model. Within 24 hours the increase of the isotope ratios from the baseline was large enough to determine the kinetic parameters. The extent of the interference and/or measurement error compared to the analytical detectability was negligible.

The pool size calculated from the spot normalized ratio at 24 hours (Table 7) was similar to the sizes derived from tri-exponential model, truncated model, and the semilogarithmic plot of the 3 - 9 day data (Table 8).

Summary:

1. Within 24 hours the extracted and unextracted plasma digestate samples gave similar results for $^{67/66}Zn$ and $^{67/68}Zn$. Therefore unextracted samples will be used the determine the Zn kinetics.

2. The sizes and turnover rates of the three consecutive pools can be measured from the long term (9 day) observations.

3. The truncated 24 hour model can be used to estimate the several kinetic parameters, including the so-called "rapidly exchangeable" 48 hour Zn pool.

4. The single 24 hour plasma sample can be used to calculate the so-called "rapidly exchangeable" 48 hour Zn pool.

5. The interferent(s) for ^{64}Zn isotope are $^{32}S_2$ and/or $^{32}S^{16}O_2$. A solution of potassium sulfate containing 287 μg sulfur/mL in 1 % nitric acid gave a signal at 64 atomic mass unit which corresponds to 11 ng Zn/mL. The correction improved the natural isotope ratio values.

Figure 1. Extracted (KY A) vs Unextracted (KY B) Zn Isotope Ratios

(a) $^{67/66}Zn$ (b) $^{67/68}Zn$

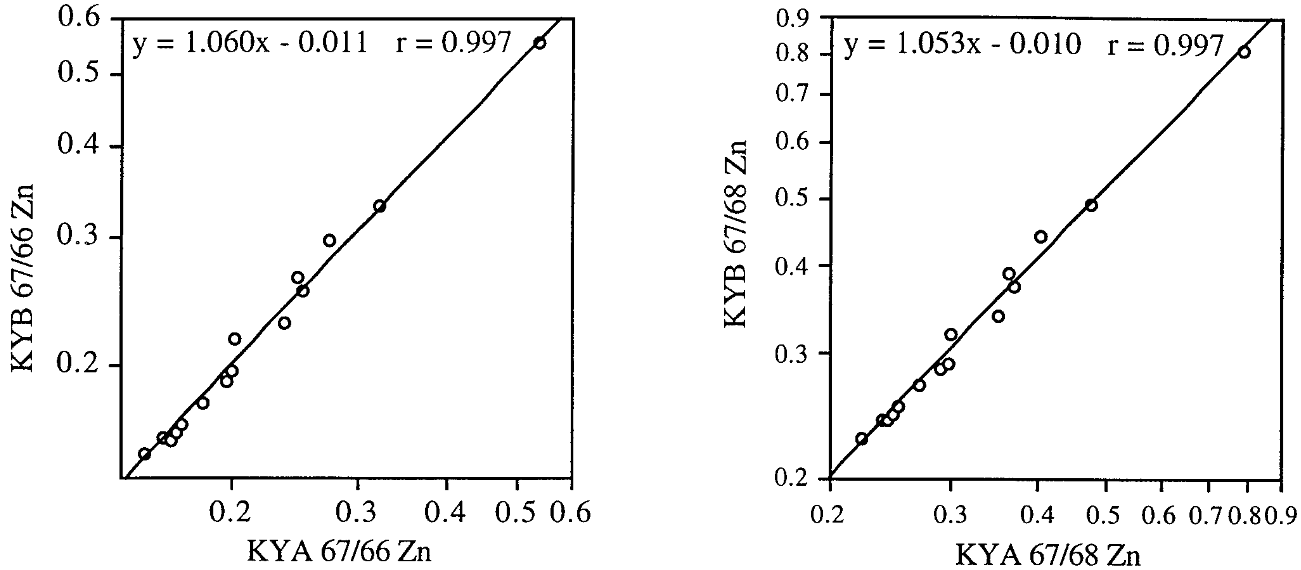


Figure 2. Disappearance Curves of Extracted Plasma (KY A) within 24 hours

(a) $^{67/66}Zn$ (b) $^{67/68}Zn$

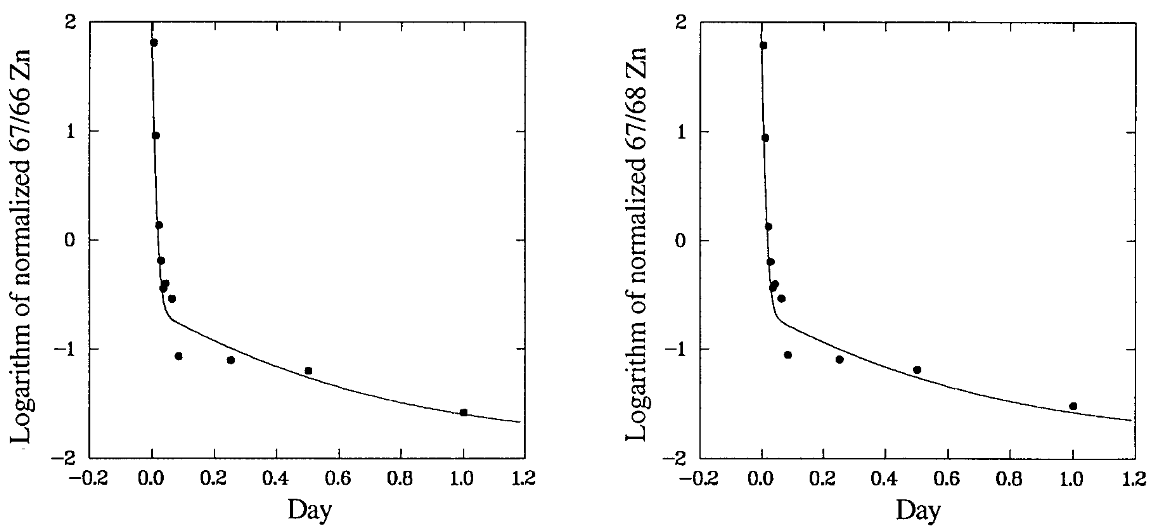
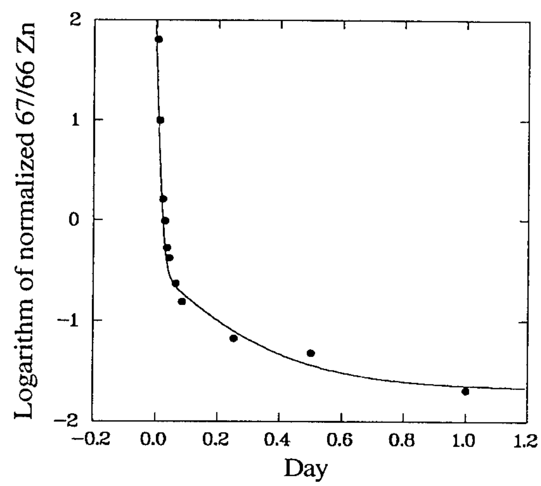


Figure 3. Disappearance Curves of Unextracted Plasma (KY B) within 24 Hours

(a) $^{67/66}Zn$



(b) $^{67/68}Zn$

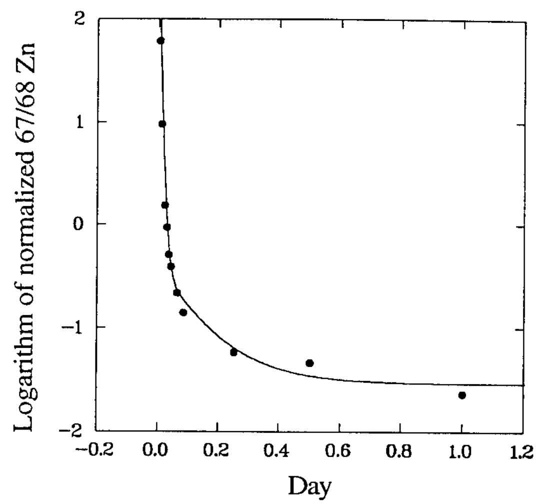


Table 1. Disappearance constant k in 30 to 60 minutes after administration (min^{-1})

Isotope ratio	KY (extracted)	KY (unextracted)	HHS (unextracted)
$^{67/66}Zn$	0.0188	0.0203	0.0200
$^{67/68}Zn$	0.0186	0.0205	0.0200

Table 2. Determined parameters using tri-exponential function models of KY and HHS

Determined parameters	KY (extracted)		KY (unextracted)		HHS (unextracted)	
	$^{67/66}Zn$	$^{67/68}Zn$	$^{67/66}Zn$	$^{67/68}Zn$	$^{67/66}Zn$	$^{67/68}Zn$
R^2	0.991	0.991	0.992	0.994	0.998	0.999
K_1	8.752	8.556	7.875	7.596	8.271	8.118
g_2	114.0	113.7	99.3	96.8	119.0	120.2
K_2	0.3342	0.3256	0.4006	0.3627	0.3918	0.3659
g_2	2.425	2.388	1.983	1.946	6.442	5.612
K_3	0.1937	0.2019	0.1302	0.1373	0.1818	0.1913
g_3	0.1242	0.1130	0.1124	0.0825	0.09986	0.1057

Table 3. Pool sizes and turnover rates of KY and HHS using tri-exponential model

Pool	KY (extracted)				KY (unextracted)				HHS (unextracted)			
	$^{67/66}Zn$		$^{67/68}Zn$		$^{67/66}Zn$		$^{67/68}Zn$		$^{67/66}Zn$		$^{67/68}Zn$	
	Size (mg)	T. R. (mg/day)	Size (mg)	T. R. (mg/day)	Size (mg)	T. R. (mg/day)	Size (mg)	T. R. (mg/day)	Size (mg)	T. R. (mg/day)	Size (mg)	T. R. (mg/da
1	4.82	550	4.93	561	5.32	529	5.53	535	5.06	602	5.16	620
2	84.8	206	84.8	203	84.3	167	89.5	174	78.0	503	80.3	451
3	231	28.7	222	25.0	344	38.6	326	26.9	246	24.6	234	24.7

TR: Turnover rate

Table 4. Determined parameters of KY and HHS using the truncated model.

Determined parameters	KY (extracted)		KY (unextracted)		HHS (unextracted)	
	$^{67/66}Zn$	$^{67/68}Zn$	$^{67/66}Zn$	$^{67/68}Zn$	$^{67/66}Zn$	$^{67/68}Zn$
R^2	0.982	0.982	0.994	0.994	0.995	0.997
K_1	8.966	8.899	8.365	8.369	8.180	8.051
g_2	117.9	120.0	108.8	112.8	116.9	118.7
K_2	0.3553	0.3520	0.4362	0.4540	0.3898	0.3732
g_2	3.367	4.246	4.260	6.607	5.412	4.823
K_3	0.2027	0.2288	0.1881	0.2150	0.1615	0.1693

Table 5. Pool sizes and turnover rates of KY and HHS obtained from $^{67/66}Zn$ using the truncated model.

Pool	KY (extracted)		KY (unextracted)		HHS (unextracted)	
	Size (mg)	Turnover rate (mg/day)	Size (mg)	Turnover rate (mg/day)	Size (mg)	Turnover rate (mg/day)
1	4.70	554	4.98	542	5.12	599
2	80.2	270	71.7	305	81.2	439
3	221	NA	238	NA	277	NA

NA: Not available since it is truncated.

Table 6. Pool sizes and turnover rates of KY and HHS obtained from $^{67/68}Zn$ using the truncated model

Pool	KY (extracted)		KY (unextracted)		HHS (unextracted)	
	Size (mg)	Turnover rate (mg/day)	Size (mg)	Turnover rate (mg/day)	Size (mg)	Turnover rate (mg/day)
1	4.72	566	4.95	558	5.21	609
2	77.0	327	66.9	442	82.5	398
3	196	NA	208	NA	264	NA

NA: Not available since it is truncated.

Table 7. Pool sizes (mg) of KY and HHS calculated from normalized isotope ratios of the 24 hour plasma sample after tracer administration

	KY (extracted)	KY (unextracted)	HHS (unextracted)
$^{67/66}Zn$	217	241	291
$^{67/68}Zn$	203	227	269

Table 8. Pool size calculated using from 3 - 9 days data of KY (extracted)

Parameters	$^{67/66}Zn$	$^{67/68}Zn$
Disappearance constant (day^{-1})	0.1187	0.1056
Pool size (mg)	240	233
Turnover rate (mg/day)	28.5	24.6

ADDENDUM II **(Prepared by NW Alcock)**

Platelets, lymphocytes and granulocytes were isolated from the blood of 9 volunteers. Zinc concentrations were measured. Problems with the cell isolation were identified. The concentration of heparin in plasma is critical. Poor recovery of platelets was caused by clumping. Increasing the amount of heparin used to 1000 units per 50 mL whole blood resulted in an excellent yield of platelets.

The zinc content of the lymphocytes, granulocytes and platelets from the 7 isolations is shown in Table 1. Also shown are the percent of cells in each suspension. The yield of granulocytes ranged from 89.5-95.9 % of the cell population. The yield of lymphocyte ranged from 61.4-91.2 % of the cell population. Contamination of the white cell fractions with platelets was insignificant. A very low zinc content in the reagent blank as determined by graphite furnace atomic absorption spectrophotometry attested to the absence of zinc contamination during the cell isolation and analytical procedure.

Table I. Zinc concentration in platelets and leukocytes and fraction purity (%)

Zinc ($\mu g/10^{10}$ cells)

Specimen	Platelets	Lymphocytes (%)**	Granulocytes (%)**
1	2.5	79.6 (63.7)	78.8 (94.4)
2	1.6	45.0 (80.4)	34.3 (95.6)
3	1.4	273.7 (91.2)	15.8 (95.9)
4	1.4	25.3 (69.6)	36.1 (89.5)
5	2.5	*---- (89.1)	62.2 (90.8)
6	7.4	161.9 (61.4)	84.2 (93.3)
7	4.2	134.0 (67.3)	79.8 (95.0)
Ref. range from literature	3.0-6.6	45.0-218	37.8-117

* A very high zinc concentration suggested contamination
** percent of total cells in the suspension identified

Beta-hydroxybutyrate stability in plasma stored at -75 degrees C is being investigated. The method of Williamson et al (1) utilizing a kit from Sigma Chemical Co. is used. It will be advantageous to perform this analysis in batches, hence the requirement for stability in plasma. Stability for two weeks at -20^0 C reported by Custer et al (2), suggests that a specimens can be stored longer at -75^0 C. Alternatively, the manual assay of Kaplan and Pesce (3) will be used.

References

1. Williamson DH, Mellanby J, Krebs HA. 1962. Enzymatic determination of D(-)beta-hydroxybutyric acid and acetoacetic acid in blood. Biochem J. 82:90

2. Custer EM, Myers JL, Poffenbarger PL, Schoen I, 1983. The storage stability of 3-hydroxybutyrate in serum, plasma and whole blood. Am J Clin Path; 80:375

3. Gau N. 1987. Beta-hydroxybutyric Acid. In Methods in Clinical Chemistry. Ed. Pesce AJ, Kaplan LA. CV Mosby Publishers, St. Louis. P101.

ADDENDUM III **(Prepared by H Dayal)**

Randomization of Subjects to Treatment Arms

The purpose of randomization is to double-blind the researchers and subjects as to the identity of the treatments. Such double-blinding is essential given the many interactions between the physician and the participants in the study.

There are two separate randomization regimens for subjects who are iron/zinc deficient and for those who are "normal". For the deficient group, the randomization is blocked by whether or not a study participant is on an oral contraceptive. Furthermore, within each subject, treatment assignments are balanced in blocks of six so that in any group of six consecutive assignment, all three treatments (Micronutrients only, Micronutrient plus iron, Micronutrient plus zinc) are equally represented. This will ensure that treatment arms will be reasonably balanced in any interim analysis.

There are only three treatments in this protocol, i.e. micronutrient only, micronutrient plus iron, and micronutrient plus zinc. However, the protocol provides for crossover after 8 weeks for the micro plus iron and the micro plus zinc arms, whereas the micro only arm is repeated in the next 8 weeks. Thus, it would be easy for the physician to identify the micro only regimen. To circumvent this problem, and to ensure double-blinding, the randomization schedule employs "aliases", whereas the other two regimens are represented by two different aliases each. This will ensure double-blinding in spite of the particular cross-over research design.

The code assignments for the treatment arms, along with alias assignment, will be kept in a locked file by the statistician. The code will be broken only for reasons of safety concerns as determined by the monitoring physician (Dr. Anderson), or for the interim and final analysis.

QUARTERLY REPORT

1. Contract No.: DAMD17-95-C-5112 2. Report Date: June 22, 1996

3. Reporting Period from: March 23, 1996 to June 22, 1996

4. PI: Harold H. Sandstead 5. Telephone No. (409) 772-4661

6. Institution: The University of Texas Medical Branch

7. Project Title: Repletion of Zinc and Iron deficiencies improves cognition of premenopausal women.

8. Current Staff, with percent effort of each on project:

Harold H. Sandstead	15 %	Nancy W. Alcock	10 %
VM Sadagopa Ramanujam	25 %	Hari H. Dayal	10 %
Norman G. Egger	90 %	Jackie Callies	60 %
Katsuhiko Yokoi	25 %		

9. Contract expenditures to date (as applicable):

	This Qtr/Cumulative		This Qtr/Cumulative
Personnel:	26,050.22 / 87,222.18	Travel:	NA / NA
Fringe Benefits:	5,952.61 / 17,384.38	Equipment:	NA / 2443.00
Supplies:	5,851.11 / 33,630.49	Other:	NA / NA

	This Qtr/Cumulative
Subtotal:	37,853.94 / 140,680.08
Indirect Costs:	17,604.14/ 56,389.67
Fee:	NA / NA
TOTAL:	**55,458.08 / 197,069.75**

10. Comments on administrative and logistical matters.

a) The supplier of the experimental treatments was unable to deliver the treatments when promised. Reasons included a lack of 'raw' materials, for example vitamin A, and a very busy production schedule. After repeated discussions and an inquiry if we should find a new supplier the production manager agreed to produce the tablets before the Fourth of July and ship them to us next week.

b) The ICP-MS was inoperable for 4 weeks. Trouble shooting resulted in cleaning all components; the photomultiplier was identified at the putative problem. The company is apparently short staffed as far as service engineers are concerned. We have been told a service call will occur the second week in July.

c) Recruitment has not proceeded rapidly. The over night stays on the CRC have prevented some women from participating. Therefore we will limit the overnight stays to phase 2, where they are necessary. Overnight stays later in the project will be optional. This increase participation.

d) A second problem has been the withholding of money from the payments to University employees. This is done on the instructions from the IRS. The University is not required to withhold money from non-employees. Therefore we will focus recruitment on non-employees. We placed an ad in the Galveston Daily News. We are negotiating placement of ads in the Houston Chronicle as a public service, the cost would otherwise be prohibitive. We will also place ads on the radio (as a public service). We are negotiating the posting advertisements on the campuses of The Galveston College, University of Houston at Clear Lake, The Texas Agricultural and Mechanical University at Galveston and the University of Houston.

11. Use additional page(s), as necessary, to describe scientific progress for the quarter in terms of the tasks or objectives listed in the statement of work for this contract. Explain deviations where this isn't possible. Include data where possible.

a) Since beginning the project eighty two (twenty seven this quarter) women were interviewed by phone; twenty six (six this quarter) were screened by medical and dietary history and laboratory methods (phase 1); thirteen (four this quarter) met the criteria for participation in phase 2, the measurement of zinc kinetics; four have completed phase 2 and nine are waiting for days 8-12 of their menstrual period, at which time they will undergo phase 2. Of the four women who completed phase 2, two displayed Zn disappearance rates in the indeterminate range, between the criteria levels that identify normal and deficient; analysis of the samples from the other two women will be done when the ICP-MS is repaired.

b) We continued to improve the method for determination of Zn kinetics and to explore relations between the various potential indicators of Zn status. Sample analysis has been completed on four of us (the investigators) and two of the subjects. The following are our findings (Tables and Figures are presented in an appendix).

The interference of $^{32}S_2$ with the measurement of ^{64}Zn was again evident, Tables 1 & 2. Therefore the normalized ratios of $^{67/66}$Zn and $^{67/68}$Zn are the most appropriate to use for calculation of zinc kinetics. As shown in Tables 1 & 2 the data from subjects AF and MC confirm the findings in two of us (KY and HHS, reported last quarter) that extracted and unextracted plasma digestate samples give similar results for $^{67/66}$Zn and $^{67/68}$Zn.

Plasma Zn isotope ratios were measured after intravenous administration of 2 mg (94 % purity) ^{67}Zn in two of us (NE and DG). Their data are included in Tables 3-6.

The disappearance constants k are shown in Table 3. The disappearance constants k of subjects AF and SM lie between our selection criteria ($k < 0.018$ or $> 0.020\ min^{-1}$) for phase 3. Therefore they are not eligible for phase 3.

The disappearance constant k of one of us (NE) was statistically detected as an outlier ($p = 0.018$). Reasons for this are unclear. He is unaware of any abnormality in metabolism. To confirm this finding representative plasma samples from his disappearance curve will be reanalyzed.

Five minute to 9 or 26 days data were analyzed by the nonlinear regression of Systat Software using a tri-exponential function model.

$$\text{Log (Normalized ratio)} = \text{Log}\,[K_1 \cdot \text{Exp}(-g_1 \cdot t) + K_2 \cdot \text{Exp}(-g_2 \cdot t) + K_3 \cdot \text{Exp}(-g_3 \cdot t)]$$

K_1:Proportional coefficient for the first term — g_1: Corresponding exponential coefficient
K_2:Proportional coefficient for the second term — g_2: Corresponding exponential coefficient
K_3:Proportional coefficient for the third term — g_3: Corresponding exponential coefficient
t: Time in days

Five minute to 24 hours data were analyzed using a truncated model.

$$\text{Log (Normalized ratio)} = \text{Log}\,[K_1 \cdot \text{Exp}(-g_1 \cdot t) + K_2 \cdot \text{Exp}(-g_2 \cdot t) + K_3]$$

To determine the influence of body size, data in Table 4 (pool sizes and turnover rates calculated from 5 min-24 hr) were adjusted for height per m^2 (Table 5) and weight per Kg (Table 6). As expected (because of smaller muscle mass) the zinc pool sizes of the two women were smaller than those of the four men. On the other hand the turnover rates, which reflect the dynamics of zinc metabolism, were similar among the men and women.

As expected the 24 hour zinc pool (pool 3) from the truncated model was highly correlated with weight, kg, and weight adjusted for height, m^2 (the body mass index, BMI) (Table 7 and Figures 1 & 2).

Pool 3 from the truncated model was highly correlated ($r = 0.981$) with the 24 hour urine creatinine excretion (Figure 3). Urinary creatinine reflects muscle mass. Thus pool 3 is in part a reflection of muscle mass. One subject (DG) indicated by the X was an outlier (studentized residual = 4.9). Likely explanations are that the ICP-MS measurement was in error, or that the ampoule of ^{67}Zn injected contained about 30 % more than was planned. To test these suppositions we will repeat the ICP-MS analysis on samples saved, and depending on the analytical findings, repeat the ^{67}Zn disappearance test.

Table 8 compares pool sizes derived by different methods of calculation. Findings derived from the kinetics models are compared with those derived from isotope dilution calculations dilution that assumes an expanding pool depending on time after the administration of the tracer. The size of pool 3 was calculated from the tri-exponential model and the truncated model. The rapidly exchangeable zinc pool size was calculated from the extrapolated intercept of the semilogarithmic plot of the normalized isotope ratio over time 3 to 9 days plasma or urine based on Miller's report (1994). With the exception of the exchangeable pool derived from the 24 hour spot urine, all values were similar. A putative reason for the apparent discrepancy is discussed below. Correlation between sizes of pool 3 derived from the truncated model and 24 hour exchangeable zinc pool derived from plasma zinc and isotope dilution calculation is shown in Figure 4. The values were almost identical. For reasons noted below this finding may be more fortuitous than physiological.

Pool sizes derived from analysis of urine collected 48 hours after administration of the isotope tended to be similar to the pool sizes derived from the various analysis of plasma. However the pool size derived from analysis of urine collected after 24 hours was not similar to the pool sized derived from the analysis of plasma collected at the same time. This finding is of interest because urinary zinc is considered to be derived from plasma and the isotope ratio of urinary zinc presumably should after 24 hours be identical to the plasma. We found the ratio of the zinc pools derived by isotope dilution calculation for the 24 hour spot urine and the 24 hour plasma zinc ranged 0.71-0.89, mean (± SD) = 0.84 (0.08), CV = 10 %. This is consistent with reports of Faure (Faure et al. 1990) and Lin (Lin et al. 1996) that the plasma exchangeable zinc fraction, i.e., *in vitro* chelatable zinc with EDTA, is about 80 % of total plasma zinc. Therefore, the 24 hour spot urine zinc provides a measure of the true exchangeable zinc, at that time interval, rather than the plasma zinc.

Based on the above relationships we calculated the "true" exchangeable zinc pool for the interval of time represented by Pool 1. We chose this time because Pool 1 is derived based on the assumption of instantaneous dilution of intravenously administered tracer in plasma or central compartment which does not require "true" exchange or chemical equilibrium with the native zinc. Thus Pool 1 represents the "total" amount of zinc in the plasma. For KY, NE, AF and SM pool 1 per body weight was 0.063±0.006 (CV 10 %) and the "exchangeable" fraction of pool 1 was 0.052±0.002 (CV 4.7 %). The CV < 5 % indicates there was little person-to-person variation in the "exchangeable" fraction.

The above applies only to the derivation of pools by isotope dilution. When the kinetic data are used to calculate pool size, the presence of an unexchangeable zinc fraction in plasma, such as zinc that is firmly bound to α 2-macroglobulin, is unimportant because the macroglobulin neither takes up or releases zinc. Thus the firmly bound and unexchangeable zinc is constant over the observation period. It gives the same "systematic error" to the proportional coefficients (K_1, K_2 and K_3) which is equivalent to the ratio of total plasma zinc to the "exchangeable" zinc. The exponential coefficients (g_1, g_2 and g_3) are not affected, as explained below:

When the isotope ratio measurement is done based on the sample which contains constant "unexchangeable" zinc fraction besides "exchangeable" zinc fraction, the measured isotope ratio will have the constant bias factor from the true isotope ratio in the "exchangeable" zinc fraction.

$$R_E = \frac{X}{E} \cdot \frac{1}{R_0} \qquad (1)$$

$$R_{E+U} = \frac{X}{E+U} \cdot \frac{1}{R_0} \qquad (2)$$

Substituting X in the equation (1) using the equation (2)

$$R_E = \frac{E+U}{E} \cdot \frac{R_0}{R} \cdot R_{E+U}$$

$$= \frac{E+U}{E} \cdot R_{E+U}$$

X : Remaining tracer isotope (^{67}Zn) after administration
E: Tracee (^{66}Zn or ^{68}Zn) isotope in the exchangeable zinc fraction
U: Tracee isotope in the unexchangeable zinc fraction

R_0: Natural isotope ratio of tracer to tracee
R_E: Normalized isotope ratio of tracer to tracee in the exchangeable zinc fraction
R_{E+U}: Normalized isotope ratio of tracer to tracee in the fraction containing exchangeable andunexchangeable zinc

Therefore, the 24 hour spot urine is a truer indicator of the 24 hour exchangeable zinc pool than the pool derived from the plasma by isotope dilution. Its relationship to pool sizes derived from kinetic data, and its utility as an indicator of zinc status remains to be determined.

References:

Faure, H. et al. (1990). Determination of the major zinc fractions in human serum by ultrafiltration. *Biological Trace Element Research* **24**, 25-37.
Lin, T. H. et al. (1996). Determination of zinc fractions in human blood and seminal plasma by ultrafiltration and atomic absorption spectrophotometry. *Biological Trace Element Research* **51**, 277-283.
Miller LV. et al. (1994) Size of the zinc pools that exchange rapidly with plasma zinc in humans: Alternative techniques for measuring and relation to dietary zinc intake. *Journal of Nutrition* 124:268-276.

c) In the chemistry laboratory, zinc is being measured in platelets, leukocytes and granulocytes. Data are shown in Table 9. The method appears to be working well. Blood samples were taken from one of us on two consecutive days, the leukocytes and platelets were isolated and their zinc concentrations determined. The results, Table 10, are consistent with good reproducibility.

Beta hydroxybutyrate has been measured in 6 of us 2 subjects (AF and SM). Comparison of the values obtained will be made at monthly intervals after storage of serum at -20°C and -70°C in order to determine whether these determinations can be made in batches rather than at the time of collection of blood from individual patients. The initial values obtained ranged from 0.69-1.98 mg/dL. The reference range for beta-hydroxybutyrate is 0-4.39 mg/dL.

12. Use additional page(s) to present a brief statement of plans or milestones for the next quarter.

Our team knows we are seriously behind schedule. We will therefore double our efforts at recruitment. We have instituted a more aggressive approach to advertising. We propose to screen sixty potential subjects and measure zinc kinetics in twenty five. We hope to begin treatment and neuropsychological testing in at least fifteen individuals. We will begin to define relationships between indices of zinc kinetics and neuropsychological function. To gain additional insights into zinc kinetics we will study four additional adult men.

APPENDIX

Table 1. Normalized isotope ratios (NIR) of Extracted vs Unextracted of Subject AF

Ratios	Slope	Intercept	R^2	P
67/64	1.0778	0.0408	0.9976	0.43×10^{-12}
67/66	0.9768	0.0486	0.9996	0.10×10^{-14}
67/68	0.9550	0.0260	0.9997	0.10×10^{-14}
67/70	0.9819	- 0.0005	0.9995	0.10×10^{-14}

NIR (Extracted) = Slope x NIR (Unextracted) + Intercept
Within 24 hours the extracted and unextracted plasma digestate samples gave similar results for $^{67/66}Zn$ and $^{67/68}Zn$.

Table 2. Normalized isotope ratios (NIR) of Extracted vs Unextracted of Subject SM

Ratios	Slope	Intercept	R^2	P
67/64	1.0373	0.2027	0.9979	0.24×10^{-12}
67/66	1.0067	0.0220	0.9998	0.10×10^{-14}
67/68	0.9976	- 0.0101	0.9999	0.10×10^{-14}
67/70	1.0213	- 0.0256	0.9998	0.10×10^{-14}

NIR (Extracted) = Slope x NIR (Unextracted) + Intercept
Within 24 hours the extracted and unextracted plasma digestate samples gave similar results for $^{67/66}Zn$ and $^{67/68}Zn$.

Table 3. Disappearance constant k (min^{-1}) calculated from 30 to 60 minutes after administration

Isotope ratio	Disppearance constant (min^{-1})	Studentized residual	P	Sigificance
HS (Male)	0.0200	-0.239	0.248	Normal
KY (Male)	0.0188	-0.458	0.155	Normal
NE (Male)	0.0325	2.034	0.018	Increased
DG (Male)	0.0188	-0.458	0.155	Normal
AF (Female)	0.0190	-0.421	0.167	Undefined
SM (Female)	0.0188	-0.458	0.155	Undefined

Except subject NE (M), plasma disappearance constants k of phase 2 were similar.
For AF and SM, k values fell within our exclusion range (0.018 - 0.020 min^{-1}).

Table 4. Pool sizes and turnover rates calculated from 5 minutes to 24 hours data

Pool	HS (M)		KY (M)		NE (M)		DG (M)		AF (F)		SM (F)	
	Size (mg)	TR (mg/day)	Size (mg)	TR (mg/day)	Size (mg)	TR (mg/day)	Size (mg)	TR (mg/day)	Size (mg)	TR (mg/day)	Size (mg)	TR (mg/day)
1	5.12	599	5.03	550	4.51	542	4.74	457	3.19	458	3.51	464
2	81.2	439	81.8	188	109	299	51.5	338	38.0	285	49.0	282
3	277	N.A.	212	N.A.	244	N.A.	183	N.A.	122	N.A.	161	N.A.

TR: Turnover rate.
N.A.: Not available since it is truncated.
Pool sizes of males were larger than females.

Table 5. Pool sizes and turnover rates per square of body height (meter^2) calculated from 5 minutes to 24 hours data

Pool	HS (M)		KY (M)		NE (M)		DG (M)		AF (F)		SM (F)	
	Size (mg/ m^2)	TR (mg/ day/m^2)	Size (mg/ m^2)	TR (mg/ day/m^2)	Size (mg/ m^2)	TR (mg/ day/m^2)	Size (mg/ m^2)	TR (mg/ day/m^2)	Size (mg/ m^2)	TR (mg/ day/m^2)	Size (mg/ m^2)	TR (mg/ day/m^2)
1	1.72	201	1.76	192	1.52	182	1.46	141	1.19	170	1.16	153
2	27.2	147	28.6	65.7	36.6	101	15.8	104	14.1	106	16.2	93.1
3	92.9	N.A.	74.1	N.A.	82.0	N.A.	56.4	N.A.	45.4	N.A.	53.2	N.A.

TR: Turnover rate.
N.A.: Not available since it is truncated.
Comparison of female subjects suggests that when the pool size per square of body height is smaller, the turnover rate per square of body height is bigger.

Table 6. Pool sizes and turnover rates per body weight calculated from 5 minutes to 24 hours data

Pool	HS (M)		KY (M)		NE (M)		DG (M)		AF (F)		SM (F)	
	Size (mg/ kg)	TR (mg/ day/kg)	Size (mg/ kg)	TR (mg/ day/kg)	Size (mg/ kg)	TR (mg/ day/kg)	Size (mg/ kg)	TR (mg/ day/kg)	Size (mg/ kg)	TR (mg/ day/kg)	Size (mg/ kg)	TR (mg/ day/kg)
1	0.0549	6.43	0.0717	7.84	0.0575	6.913	0.0614	5.92	0.0627	9.00	0.0586	7.75
2	0.871	4.71	1.17	2.68	1.39	3.81	0.667	4.37	0.747	5.60	0.818	4.71
3	2.97	N.A.	3.02	N.A.	3.11	N.A.	2.37	N.A.	2.40	N.A.	2.69	N.A.

TR: Turnover rate.
N.A.: Not available since it is truncated.
The size of pool 3 per body weight of DG was similar to the female subjects rather than to another male subjects.

Table 7. Correlation matrix among anthropometrics and zinc kinetic parameters. The upper triangle shows correlation coefficients and the lower triangle shows P values.

	Body weight	Body height	BMI	Pool 1	TR 1	Pool 2	TR 2	Pool 3
Body weight	-	0.527	**0.966**	**0.874**	0.757	0.635	0.635	**0.933**
Body height	0.283	-	0.290	0.438	-0.090	0.080	0.365	0.297
BMI	**0.002**	0.578	-	**0.856**	**0.890**	0.721	0.587	**0.968**
Pool 1	**0.023**	0.385	**0.030**	-	0.734	0.650	0.235	**0.836**
TR 1	0.081	0.865	**0.018**	0.097	-	0.787	0.299	**0.912**
Pool 2	0.162	0.880	0.106	0.163	0.063	-	0.036	**0.842**
TR 2	0.176	0.477	0.221	0.655	0.565	0.945	-	0.456
Pool 3	**0.007**	0.568	**0.002**	**0.038**	**0.011**	**0.036**	0.364	-

TR: Turnover rate.
We found high positive correlation ($r > 0.8$, $P < 0.05$) between pools 1 and 3 and body weight or BMI.

Table 8. Comparison of zinc pool sizes calculated by different methods

Method	HS (M)	KY (M)	NE (M)	DG (M)	AF (F)	SM (F)
1: Pool 3 from tri-exponential model (5 min - 9 d)	222	220	228	187	-	-
2: Pool 3 from truncated model (5 min - 24 h)	250	213	244	183	122	161
3: Rapidly exchangeable pool from 3- 9 day plasma	-	228	246	208	-	-
4: Exchangeable pool based on 24 h plasma	256	206	228	189	128	164
5: Rapidly exchangeable pool from 3- 9 day urine	-	193	250	- -	-	-
6: Exchangeable pool based on 2 day spot urine	260	203	237	- -	-	213
7: Exchangeable pool based on 1 day spot urine	-	147	195	- -	112	144
Ratio of 1 day urine pool (7) to 24 h plasma pool (4)	-	0.71	0.86	- -	0.88	0.89

All values derived from various methods were similar.
- indicates sample not collected from the subject.
- - indicates sample not analyzed yet.

Figure 1. Pool 3 vs Body weight

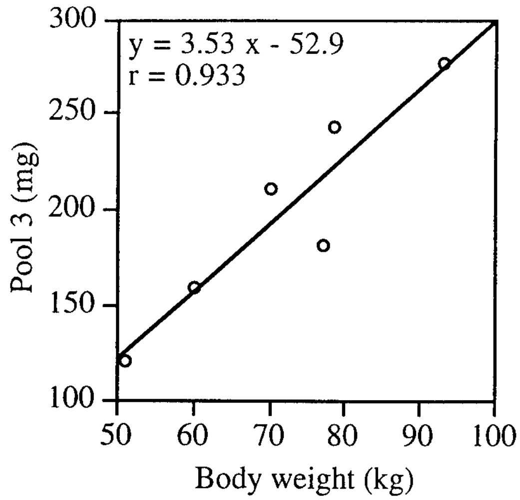
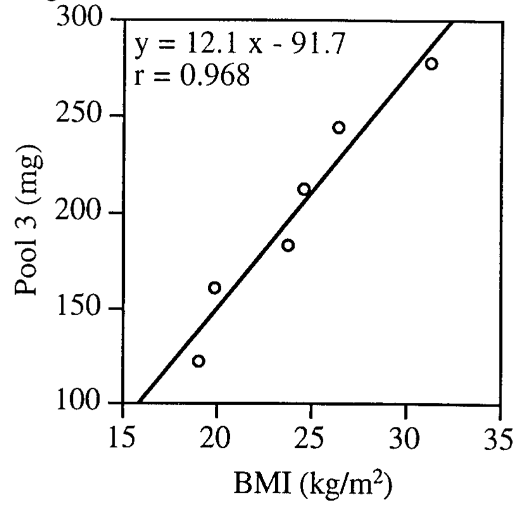
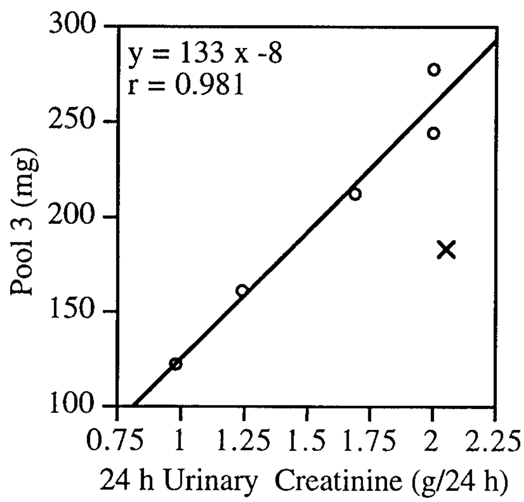


Figure 2. Pool 3 vs BMI



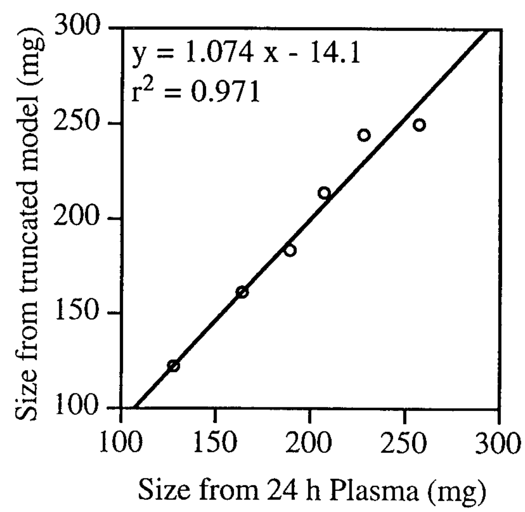
High positive correlation was found between pool 3 and body weight (Figure 1) or BMI (Figure 2).

Figure 3. Pool 3 calculated from trunacted modelvs 24 h urinary creatinine excretion



Pool 3 correlated well with 24 h urinary creatinine excretion.

Figure 4. Size of pool 3 calculated from the truncated model and single 24 hour plasma



A single 24 hour plasma can be an estimator of the so-called "rapidly exchangeable" 48 hour Zn pool.

Table 9. Zinc concentration in platelets and leukocytes and fraction purity (%)

Zinc ($\mu g/10^{10}$ cells)

Specimen	Platelets	Lymphocytes (%)**	Granulocytes (%)**
1	2.5	79.6 (63.7)	78.8 (94.4)
2	1.6	45.0 (80.4)	34.3 (95.6)
3	1.4	273.7 (91.2)	15.8 (95.9)
4	1.4	25.3 (69.6)	36.1 (89.5)
5	2.5	*---- (89.1)	62.2 (90.8)
6	7.4	161.9 (61.4)	84.2 (93.3)
7	4.2	134.0 (67.3)	79.8 (95.0)
8	4.2	68.1 (64.5)	42.1 (96.4)
9	7.6	106.8 (79.8)	241.4 (94.7)
10	4.5	122.0 (72)	49.8 (94.1)
11	6.9	148.5 (68.3)	146.0 (87.8)
AF	6.4	103.5 (66.8)	138.9 (94.2)
SM	3.4	113.3 (63.2)	36.5 (92.8)
DH	5.2	59.2 (66.3)	45.5 (-)
Ref. range from literature	3.0-6.6	45.0-218	37.8-117

Previous Data

* A very high zinc concentration indicated possible contamination

** % of total cells in suspension identified

AF, SM, DH - study subjects

The purity of the cell fractions from isolation to isolation is consistent.

Table 10. Blood samples were taken from one volunteer on two consecutive days, the leukocytes and platelets isolated and their zinc concentration determined. The following results were obtained:

$\mu g/Zn/10^{10}$cells

Sample Date	Platelets	Lymphocytes	Granulocytes
5/6/96	4.4	113.7	53.1
5/7/96	4.6	131.8	46.5

The results indicated good reproducibility of the lengthy isolation and subsequent zinc analysis.

Demographic and Health Status Questionnaire

Date:___________ Patient number________

Last Name:_________________________ First Name:____________________ Initial____
Address: ____
____

Home Phone:___________ Business Phone:____________

Date of Birth:month/day/year ___/___/___ Country of Birth______________________
Your current age________ years.

- What is your ethnicity (race)? Please circle one.

Black **White** **Hispanic** **Asian** **Indian** **Other**

- What is your marital status? Please circle one.

Single **Married** **Divorced** **Widowed**

- What is the highest level of education you had? Please circle one.

Less than High School **High School diploma** **Some College**
College graduate **Masters Degree** **Doctorate Degree**

- Are you employed? Please circle one. **Yes** **No**

If yes, what is your Job title?____

- What is your total household income per year? Please circle one.

Less than $ 14.350
$ 14.350 - $ 25.000
$ 25.001 - $ 50.000
More than $ 50.000

- Who can we notify in case of emergency?.

Name____
Address____
This person is your (circle one):

Spouse **Mother** **Father** **Brother** **Sister**

Child **Friend** **Employer** **Collegue** **Neighbor**

Current Medical Problems (symptoms)

- Do you have any of the following symptoms (now or within the last 3 months)? Please indicate this by circling either Yes or No.

Headache	Yes	No
Fatigue	Yes	No
Dizziness	Yes	No
Difficulty with sleeping	Yes	No
Depression	Yes	No
Fever	Yes	No
Pain anywhere	Yes	No
Cough	Yes	No
Asthma	Yes	No
Running nose	Yes	No
Shortness of breath	Yes	No
Chest pain	Yes	No
Swollen legs	Yes	No
Weightloss more then 10 lb (last 6 months)	Yes	No
Loss of appetite	Yes	No
Diarrhea	Yes	No
Constipation	Yes	No
Black stools	Yes	No
Blood in your stools	Yes	No
Frequent urination	Yes	No
Painful urination	Yes	No
Blood in urine	Yes	No
Easy bruising	Yes	No
Pain in your joints	Yes	No
Swollen joints	Yes	No

- Do you have any allergies? Please circle one. **Yes** **No**
  If yes, please write any allergies you have below.

- Do you have other symptoms or medical conditions?
  Please circle one. **Yes** **No**
  If yes, please write any other current symptoms or medical conditions below.

- Do you take any medication(s)? Please circle one. **Yes** **No**
  If yes, please write medication(s) you take and the date you started taking these medications.

Medication	**start date** (day/ month/ year)
______________	___/___/___
______________	___/___/___
______________	___/___/___
______________	___/___/___
______________	___/___/___
______________	___/___/___

- Do you take nutrition supplements (vitamins and other)?
  Please circle one. **Yes** **No**
  If yes, please write supplements you take and the date you started taking these supplements

Supplement	**start date** (day/ month/ year)
______________	___/___/___
______________	___/___/___
______________	___/___/___
______________	___/___/___

- Do you drink alcohol in any form? Please circle one. **Yes** **No**
  If yes, please write the number of drinks you have per week below.
  ________per week.

- Do you smoke currently ? Please circle one. **Yes** **No**
  If yes, please write the number of cigarettes you smoke per day below.
  ________per day.

PAST MEDICAL HISTORY

Has a doctor diagnosed any of the following conditions or diseases in the past?
Please indicate this by circling either Yes or No.

Neurological disease (epilepsy, muscle weakness)	**Yes**	**No**
Lung disease (asthma, bronchitis, tuberculosis)	**Yes**	**No**
Heart and Vessel disease (murmurs, hypertension, thrombosis)	**Yes**	**No**
Gastrointestinal disease (ulcers, jaundice, hepatitis, gallstones)	**Yes**	**No**
Kidney or Bladder disease (infections, stones)	**Yes**	**No**
Rheumatological disease (arthritis, lupus)	**Yes**	**No**
Endocrinological disease (diabetes, thyroid)	**Yes**	**No**
Psychiatric diseases (eating disorders, depression, anxiety)	**Yes**	**No**
Hematological disease (anemia, bleeding disorder, sickle cell disease)	**Yes**	**No**
Orthopedic disease (fractures, osteoporosis, hernias, bone malformations)	**Yes**	**No**

GYNECOLOGICAL AND MENSTRUAL HISTORY

For this study your Gynecological and Menstrual history is very important. We need therefore some more detailed information.

When responding to the following questions concerning your menstruation, consider only the *last two years*. To avoid misunderstanding between menstrual *period* and menstrual *cycle* we have defined these as follows:
"Menstrual period": Bleeding for 2 or more consecutive days; bleeding for a single day is considered "break-through bleeding".
"Menstrual cycle": Time (in days) between the *first* day of bleeding for one menstrual period to the *first* day of bleeding for the next menstrual period.

- What was your age at the time of your *first* menstrual period? __________ years.
- When was the *first day* of your *last* period? (month/day/year)_____/_____/____
- Have you ever been treated for a menstrual related disorder such as premenstrual tension, excessive pain, heavy flow? Please circle one. **Yes No**
- Do you menstruate regularly? (In other words; are your periods approximately the same number of days apart each cycle?). Please circle one. **Yes No**
- On average how long is your menstrual cycle? (how many *days* are there between the *start* of one menstrual period and the *start* of the next)____________days.
- How long is your menstrual period (how many *days* is your flow).__________days.
- How would you consider your typical menstrual flow?
  - **light:** Please circle one. **Yes No**
  - **moderate:** Please circle one. **Yes No**
  - **heavy:** Please circle one. **Yes No**
- Do you ever bleed through the menstrual pad? Please circle one. **Yes No**
- Did you skip or miss more than one period in the last two years? Please circle one. **Yes No**
- How many times a *year* do you experience break-through bleeding (bleeding for a single day)?_________times/year.
- Have you ever taken medication for relief of symptoms associated with menstruation or your menstrual cycle? Please circle one. **Yes No**
- What kind of symptoms (physical, emotional, or other) do you experience in relation to your menstrual cycle? (include symptoms occurring the week prior to and during the days of blood flow).

  ____

  ____
- Have you ever been pregnant ? (Please include pregnancies which were less than full term and miscarriages). Please circle one. **Yes No**
  If yes, how many times have you been pregnant?_________times.
  how many of these pregnancies were miscarriages?________
  when was your last pregnancy? (month/day/year)_____/_____/____
- Are you now pregnant? Please circle one. **Yes No**
- Are you taking currently or have you taken within the last 3 months birth control pills? Please circle one. **Yes No**
- Do you have currently or have you had within the last 3 months an intra uterine device ? Please circle one. **Yes No**
- Have you ever been treated for a gynecological problem such as infections, tumors, infertility. Please circle one. **Yes No**
- Have you ever had a gynecological operation such as ovaries or uterus removed, tubal ligation? Please circle one. **Yes No**

PERMISSION TO CONTACT PHYSICIAN

We need your written permission to contact your physician. Please complete the following questions and sign and date this page.

- Who is your Physician?

Name____

Address:____

Phone number____

- Do you give permission to notify your physician in case of emergency?.

Please circle one. **Yes** **No**

- Do you give permission to obtain medical information from your physician in case it is needed for better evaluation of your medical history?

Please circle one. **Yes** **No**

- Would you like us to send your study results to your physician?.

Please circle one. **Yes** **No**

Your Name____

Your Signature_______________________ **Date**___________________

COMMENTS TO BE FILLED OUT BY INTERVIEWER.

Please comment on any "yes" response

Questions ______________________________

Comments ______________________________

____

Questions ______________________________

Comments ______________________________

____

Questions ______________________________

Comments ______________________________

____

Questions ______________________________

Comments ______________________________

____

Questions ______________________________

Comments ______________________________

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Questions ______________________________

Comments ______________________________

____

Questions ______________________________

Comments ______________________________

____

Date of Interview: ______________

Name of Interviewer: ______________________________

Form HQ: 12/16/95

Food Intake Questionnaire

Please check the appropriate box.

Date: ______________________

Subject name: ______________________________________

No. ______________

Screening ☐ Baseline ☐ Crossover ☐ End of Study ☐

How often do you usually eat the following foods? Consider the past month only.

	3 or more per day	2 per day	1 per day	5 per week	2-4 per week	1 per week	Occasionally	Never	Don't Know
Non-Alcoholic Beverages									
Hi-C, Tang drinks	☐	☐	☐	☐	☐	☐	☐	☐	☐
Regular or decaf coffee	☐	☐	☐	☐	☐	☐	☐	☐	☐
Regular, decaf or herbal tea	☐	☐	☐	☐	☐	☐	☐	☐	☐
Tomato or V8 Juice	☐	☐	☐	☐	☐	☐	☐	☐	☐
Alcoholic Beverages									
Beer and Lite Beer	☐	☐	☐	☐	☐	☐	☐	☐	☐
Wine, wine coolers, sangria and champagne	☐	☐	☐	☐	☐	☐	☐	☐	☐
Hard liquor: tequila, gin, vodka, scotch, rum ...	☐	☐	☐	☐	☐	☐	☐	☐	☐
Vegetables (raw, frozen, canned, cooked)									
Carrots and vegetable mixtures w/ carrots	☐	☐	☐	☐	☐	☐	☐	☐	☐

Food Intake Questionnaire

Please check the appropriate box.

Date: ____________

Subject name: ____________

No. ____________

Screening ☐ Baseline ☐ Crossover ☐ End of Study ☐

How often do you usually eat the following foods? Consider the past month only.

	3 or more per day	2 per day	1 per day	5 per week	2-4 per week	1 per week	Occasionally	Never	Don't Know
Broccoli, brussel sprouts, cabbage, cole slaw and cauliflower	☐	☐	☐	☐	☐	☐	☐	☐	☐
Potatoes, including baked, mashed, boiled etc.	☐	☐	☐	☐	☐	☐	☐	☐	☐
Sweet potatoes	☐	☐	☐	☐	☐	☐	☐	☐	☐
Tomatoes	☐	☐	☐	☐	☐	☐	☐	☐	☐
Spinach, greens, collards and kale	☐	☐	☐	☐	☐	☐	☐	☐	☐
Lettuce	☐	☐	☐	☐	☐	☐	☐	☐	☐
Hot red chili peppers-not ground red peppers (green, yellow)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Squash	☐	☐	☐	☐	☐	☐	☐	☐	☐
Mushrooms	☐	☐	☐	☐	☐	☐	☐	☐	☐

Food Intake Questionnaire

Please check the appropriate box. Date: ______________

Subject name: ______________________________ No. ____________

Screening ☐ Baseline ☐ Crossover ☐ End of Study ☐

How often do you usually eat the following foods? Consider the past month only.

	3 or more per day	2 per day	1 per day	5 per week	2-4 per week	1 per week	Occasionally	Never	Don't Know
Corn	☐	☐	☐	☐	☐	☐	☐	☐	☐
Green beans	☐	☐	☐	☐	☐	☐	☐	☐	☐
Green peas	☐	☐	☐	☐	☐	☐	☐	☐	☐
Beans, Nuts, Cereals, Grains									
Beans-kidney, refried, pinto, baked, lentils, etc	☐	☐	☐	☐	☐	☐	☐	☐	☐
Peanut, peanut butter, other nuts and seeds	☐	☐	☐	☐	☐	☐	☐	☐	☐
All bran, all bran x-tra - 100%, Bran, Fiber One	☐	☐	☐	☐	☐	☐	☐	☐	☐
Total Product 19, Most and Just Right	☐	☐	☐	☐	☐	☐	☐	☐	☐
All other cold cereals-Cherrios etc.	☐	☐	☐	☐	☐	☐	☐	☐	☐
Cooked hot cereals-cream of wheat etc.	☐	☐	☐	☐	☐	☐	☐	☐	☐

Food Intake Questionnaire

Please check the appropriate box. Date: ______________

Subject name: ______________________________ No. __________

Screening ☐ Baseline ☐ Crossover ☐ End of Study ☐

How often do you usually eat the following foods? Consider the past month only.

	3 or more per day	2 per day	1 per day	5 per week	2-4 per week	1 per week	Occasionally	Never	Don't Know
White bread, rolls, bagels, biscuits, muffins (sandwiches)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Dark breads and rolls-whole wheat, rye, pumpernickel	☐	☐	☐	☐	☐	☐	☐	☐	☐
Corn tortillas	☐	☐	☐	☐	☐	☐	☐	☐	☐
Corn bread and muffins	☐	☐	☐	☐	☐	☐	☐	☐	☐
Flour tortillas	☐	☐	☐	☐	☐	☐	☐	☐	☐
White rice	☐	☐	☐	☐	☐	☐	☐	☐	☐
Brown Rice	☐	☐	☐	☐	☐	☐	☐	☐	☐
Main Dishes: meat, fish, chicken and eggs									
Any type of stew or soup containing red meat	☐	☐	☐	☐	☐	☐	☐	☐	☐

Food Intake Questionnaire

Please check the appropriate box. Date: ____________

Subject name: ______________________ No. ________

Screening ☐ Baseline ☐ Crossover ☐ End of Study ☐

How often do you usually eat the following foods? Consider the past month only.

	3 or more per day	2 per day	1 per day	5 per week	2-4 per week	1 per week	Occasionally	Never	Don't Know
Spaghetti and pasta or other pasta with red meat	☐	☐	☐	☐	☐	☐	☐	☐	☐
Any type of stew or soup containing chicken	☐	☐	☐	☐	☐	☐	☐	☐	☐
Spaghetti and pasta or other pasta with chicken	☐	☐	☐	☐	☐	☐	☐	☐	☐
Any type of stew or soup containing seafood	☐	☐	☐	☐	☐	☐	☐	☐	☐
Spaghetti and pasta or other pasta with seafood	☐	☐	☐	☐	☐	☐	☐	☐	☐
Bacon, sausage (Chorizo) and luncheon meats-hotdogs, bologna	☐	☐	☐	☐	☐	☐	☐	☐	☐
Liver and other organ meats	☐	☐	☐	☐	☐	☐	☐	☐	☐
Beef-hamburger, steaks, roast beef, meatloaf	☐	☐	☐	☐	☐	☐	☐	☐	☐

Food Intake Questionnaire

Please check the appropriate box.

Date: ____________

Subject name: ____________

No. ____________

Screening ☐ Baseline ☐ Crossover ☐ End of Study ☐

How often do you usually eat the following foods? Consider the past month only.

	3 or more per day	2 per day	1 per day	5 per week	2-4 per week	1 per week	Occasionally	Never	Don't Know
Pork and ham, roast pork, pork chop, ribs	☐	☐	☐	☐	☐	☐	☐	☐	☐
Lamb, sheep goat	☐	☐	☐	☐	☐	☐	☐	☐	☐
Clams, oysters, snails	☐	☐	☐	☐	☐	☐	☐	☐	☐
Shrimp, crab, lobster	☐	☐	☐	☐	☐	☐	☐	☐	☐
Fish-fillets, fish sticks, sandwiches and tuna	☐	☐	☐	☐	☐	☐	☐	☐	☐
Chicken-baked, fried, nuggets, salads(turkey)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Eggs-scrambled, fried, omelets, boiled, salad	☐	☐	☐	☐	☐	☐	☐	☐	☐
Milk and Milk Products (not used in cooking)									
Yogurt and frozen yogurt	☐	☐	☐	☐	☐	☐	☐	☐	☐
A glass of milk	☐	☐	☐	☐	☐	☐	☐	☐	☐

Food Intake Questionnaire

Please check the appropriate box.

Date: ____________

Subject name: ____________

No. ____________

Screening ☐ Baseline ☐ Crossover ☐ End of Study ☐

How often do you usually eat the following foods? Consider the past month only.

	3 or more per day	2 per day	1 per day	5 per week	2-4 per week	1 per week	Occasionally	Never	Don't Know
Milk added to cereals and other items. (Not coffee or tea)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Chocolate milk and cocoa	☐	☐	☐	☐	☐	☐	☐	☐	☐
Sour cream	☐	☐	☐	☐	☐	☐	☐	☐	☐
Ice cream, milkshakes	☐	☐	☐	☐	☐	☐	☐	☐	☐
Cheese, all types (swiss, american, cottage)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Cheese containing pasta dishes-macaroni	☐	☐	☐	☐	☐	☐	☐	☐	☐
Cheese in pizza, calzone or lasagna	☐	☐	☐	☐	☐	☐	☐	☐	☐
Cheese nachos, enchiladas, quesadillas	☐	☐	☐	☐	☐	☐	☐	☐	☐
Fruit and fruit juices (frozen, canned, dried)									
Orange juice, grapefruit juice	☐	☐	☐	☐	☐	☐	☐	☐	☐

Food Intake Questionnaire

Please check the appropriate box. Date: ____________

Subject name: ____________________ No. ________

Screening ☐ Baseline ☐ Crossover ☐ End of Study ☐

How often do you usually eat the following foods? Consider the past month only.

	3 or more per day	2 per day	1 per day	5 per week	2-4 per week	1 per week	Occasionally	Never	Don't Know
Mixed fruit juices	☐	☐	☐	☐	☐	☐	☐	☐	☐
Other fruit juices-grape, apple, cranberry etc.	☐	☐	☐	☐	☐	☐	☐	☐	☐
Citrus fruits-oranges, grapefruit, tangerine, kiwi, strawberry	☐	☐	☐	☐	☐	☐	☐	☐	☐
Melon-cantaloupe, honey dew, watermelon	☐	☐	☐	☐	☐	☐	☐	☐	☐
Peaches, nectarines, apricots, guava, mango, papaya	☐	☐	☐	☐	☐	☐	☐	☐	☐
Other fruits-apple, banana, pear, berry, cherry, grapes, plums	☐	☐	☐	☐	☐	☐	☐	☐	☐
Have we missed any other foods or beverages you had in the past month?									
Food/Beverage									
____________	☐	☐	☐	☐	☐	☐	☐	XXX	XXX
____________	☐	☐	☐	☐	☐	☐	☐	XXX	XXX

Food Intake Questionnaire

Please check the appropriate box.

Date: ____________________

Subject name: ________________________________

No. ______________

Screening ☐ Baseline ☐ Crossover ☐ End of Study ☐

How often do you usually eat the following foods? Consider the past month only.

	3 or more per day	2 per day	1 per day	5 per week	2-4 per week	1 per week	Occasionally	Never	Don't Know
______	☐	☐	☐	☐	☐	☐	☐	XXX	XXX
______	☐	☐	☐	☐	☐	☐	☐	XXX	XXX
______	☐	☐	☐	☐	☐	☐	☐	XXX	XXX

Appendix II

Preventative Medicine and Community Health
Division of Human Nutrition
700 Harborside Drive
Ewing Building, Room 3.102
University of Texas Medical Branch
Galveston Texas, 77555-1109
(409) 772-4661

MDQ Booklet
Zinc & Iron in Premenopausal Women

Sandstead/Egger/Penland

Participant # __________

Distributed on: _____/_____/_____

United States Department of Agriculture
Agricultural Research Service
Northern Plains Area
Grand Forks Human Nutrition Research Center
2420 2nd Avenue N.
P.O. Box 9034
Grand Forks, North Dakota
58202-9034
Fax (701) 795-8395

Dear Study Participant:

The following pages each contain a list of symptoms which some women have identified as occurring in relation to their menstrual cycle. We are interested in determining whether the occurrence and severity of these symptoms are affected by the dietary treatments you receive during this study. Previous research has suggested that dietary iron may affect menstrual symptomatology. Therefore, were are asking you to report any symptoms you might experience each day, and indicate their severity.

Instructions for completing the forms in this booklet:
This booklet contains 15 identical forms. One form should be completed each day. If you look at the form, you will notice that item #s 7, 13, 16, 23, 43 and 52 are printed in bold letters. Each of these items concern your sleep. Please complete these 6 items within 30 minutes of waking each morning. Complete all other items and answer the two questions at the bottom of the form at the end of the day, within 30 minutes of when you retire to bed for the night. Also, please write the time you complete the form in the evening in the space provided in the upper right corner of the form. Do not refer to your responses on previous days when completing the form. Please conscientiously report symptoms whether or not you believe they are related to your menstrual cycle. Past experience indicates that the form requires approximately 5 minutes to complete.

You should complete the first form in the booklet seven (7) days prior to the expected start date of your menstrual period (bleeding). For example, if you know you will begin your period on the 8th of the month, you should begin completing forms in this booklet on the 1st of the month.

We greatly appreciate your participation in this study as a whole, and in this particular phase of the study. We believe the information gathered will be important to better understanding the role of nutrition in women's health and sense of well-being.

If you have any questions or concerns regarding completing the forms in this booklet, please contact:

Jackie Callies
Preventative Medicine and Community Health
Division of Human Nutrition
700 Harborside Drive
Ewing Building, Room 3.102
University of Texas Medical Branch
Galveston Texas, 77555-1109
(409) 772-4661

Once again, thank you for your participation!

Sincerely,

James G. Penland

James G. Penland, Ph.D.
Research Psychologist

Subject No. __________ Today's Date ____ / ____ / ____ Time Completed ____ : ____

Please darken the appropriate circle next to *each* symptom listed below to describe your experience during the past 24 hours (today). Items in **bold print** (#s 7,13,16,23,43 and 52) may be completed immediately upon awakening in the morning; all other items should be completed immediately prior to bedtime. You may use either a pen or pencil.

Respond to each symptom as: (N)ot present; (P)resent, mild; (M)oderate; (S)trong; or, (A)cute, partially disabling.

N	P	M	S	A		N	P	M	S	A	
○	○	○	○	○	1. Nausea or vomiting	○	○	○	○	○	31. Daytime sleepiness
○	○	○	○	○	2. Loneliness	○	○	○	○	○	32. Weight gain
○	○	○	○	○	3. Hot flashes (upper body warmth/heat)	○	○	○	○	○	33. Painful or tender breasts
○	○	○	○	○	4. Anxiety or tension	○	○	○	○	○	34. Feelings of anger or rage
○	○	○	○	○	5. Numbness or tingling in hands or feet	○	○	○	○	○	35. Difficulty making decisions/solving problems
○	○	○	○	○	6. Cramps (uterine or pelvic)	○	○	○	○	○	36. Bursts of energy or activity
○	○	○	○	○	**7. Insomnia (difficulty getting to sleep)**	○	○	○	○	○	37. Loss of confidence or self-esteem
○	○	○	○	○	8. Difficulty concentrating (distractible)	○	○	○	○	○	38. Worrying
○	○	○	○	○	9. Shortness of breath	○	○	○	○	○	39. Swelling (breasts, abdomen, ankles, etc.)
○	○	○	○	○	10. Dizziness or faintness	○	○	○	○	○	40. Tiredness or fatigue
○	○	○	○	○	11. Depression (feeling sad or blue)	○	○	○	○	○	41. Increased need for order (orderliness)
○	○	○	○	○	12. Digestive problems	○	○	○	○	○	42. Mood swings
○	○	○	○	○	**13. Night sweats**	○	○	○	○	○	**43. Poor sleep quality**
○	○	○	○	○	14. Feelings of fear or panic	○	○	○	○	○	44. Frequent urination
○	○	○	○	○	15. Chest pains	○	○	○	○	○	45. Decreased efficiency or lowered performance
○	○	○	○	○	**16. Night-time awakenings**	○	○	○	○	○	46. Cold shivers
○	○	○	○	○	17. Memory problems (forgetfulness)	○	○	○	○	○	47. Poor motor coordination
○	○	○	○	○	18. Skin tingling/crawling or feels prickly	○	○	○	○	○	48. Avoiding social activities
○	○	○	○	○	19. Loss of interest in usual activities	○	○	○	○	○	49. Cravings for specific foods
○	○	○	○	○	20. Clumsiness or increased accidents	○	○	○	○	○	50. Irritability
○	○	○	○	○	21. Palpitations (heart pounding)	○	○	○	○	○	51. Feelings of suffocation
○	○	○	○	○	22. Muscle stiffness	○	○	○	○	○	**52. Nightmares**
○	○	○	○	○	**23. Early-morning awakenings**	○	○	○	○	○	53. Vision or hearing problems
○	○	○	○	○	24. Poor judgment	○	○	○	○	○	54. Feelings of frustration
○	○	○	○	○	25. Backache	○	○	○	○	○	55. Hopelessness
○	○	○	○	○	26. Reduced sense of enjoyment	○	○	○	○	○	56. Cold sweats
○	○	○	○	○	27. Change in appetite	○	○	○	○	○	57. Disorientation or confusion
○	○	○	○	○	28. Restlessness	○	○	○	○	○	58. Flushing (chest, neck and face)
○	○	○	○	○	29. Headache	○	○	○	○	○	59. General aches and pains (joint, muscle, etc.)
○	○	○	○	○	30. Crying	○	○	○	○	○	60. Reduced sense of well-being

Did you menstruate or experience break-through bleeding today? Yes No (circle one)

Did you take any medications today? Yes No (circle one)

Subject No. __________ Today's Date ____ / ____ / ____ Time Completed ____ : ____

Please darken the appropriate circle next to *each* symptom listed below to describe your experience during the past 24 hours (today). Items in **bold print** (#s 7,13,16,23,43 and 52) may be completed immediately upon awakening in the morning; all other items should be completed immediately prior to bedtime. You may use either a pen or pencil.

Respond to each symptom as: (N)ot present; (P)resent, mild; (M)oderate; (S)trong; or, (A)cute, partially disabling.

N	P	M	S	A	Symptom	N	P	M	S	A	Symptom
○	○	○	○	○	1. Nausea or vomiting	○	○	○	○	○	31. Daytime sleepiness
○	○	○	○	○	2. Loneliness	○	○	○	○	○	32. Weight gain
○	○	○	○	○	3. Hot flashes (upper body warmth/heat)	○	○	○	○	○	33. Painful or tender breasts
○	○	○	○	○	4. Anxiety or tension	○	○	○	○	○	34. Feelings of anger or rage
○	○	○	○	○	5. Numbness or tingling in hands or feet	○	○	○	○	○	35. Difficulty making decisions/solving problems
○	○	○	○	○	6. Cramps (uterine or pelvic)	○	○	○	○	○	36. Bursts of energy or activity
○	○	○	○	○	**7. Insomnia (difficulty getting to sleep)**	○	○	○	○	○	37. Loss of confidence or self-esteem
○	○	○	○	○	8. Difficulty concentrating (distractible)	○	○	○	○	○	38. Worrying
○	○	○	○	○	9. Shortness of breath	○	○	○	○	○	39. Swelling (breasts, abdomen, ankles, etc.)
○	○	○	○	○	10. Dizziness or faintness	○	○	○	○	○	40. Tiredness or fatigue
○	○	○	○	○	11. Depression (feeling sad or blue)	○	○	○	○	○	41. Increased need for order (orderliness)
○	○	○	○	○	12. Digestive problems	○	○	○	○	○	42. Mood swings
○	○	○	○	○	**13. Night sweats**	○	○	○	○	○	**43. Poor sleep quality**
○	○	○	○	○	14. Feelings of fear or panic	○	○	○	○	○	44. Frequent urination
○	○	○	○	○	15. Chest pains	○	○	○	○	○	45. Decreased efficiency or lowered performance
○	○	○	○	○	**16. Night-time awakenings**	○	○	○	○	○	46. Cold shivers
○	○	○	○	○	17. Memory problems (forgetfulness)	○	○	○	○	○	47. Poor motor coordination
○	○	○	○	○	18. Skin tingling/crawling or feels prickly	○	○	○	○	○	48. Avoiding social activities
○	○	○	○	○	19. Loss of interest in usual activities	○	○	○	○	○	49. Cravings for specific foods
○	○	○	○	○	20. Clumsiness or increased accidents	○	○	○	○	○	50. Irritability
○	○	○	○	○	21. Palpitations (heart pounding)	○	○	○	○	○	51. Feelings of suffocation
○	○	○	○	○	22. Muscle stiffness	○	○	○	○	○	**52. Nightmares**
○	○	○	○	○	**23. Early-morning awakenings**	○	○	○	○	○	53. Vision or hearing problems
○	○	○	○	○	24. Poor judgment	○	○	○	○	○	54. Feelings of frustration
○	○	○	○	○	25. Backache	○	○	○	○	○	55. Hopelessness
○	○	○	○	○	26. Reduced sense of enjoyment	○	○	○	○	○	56. Cold sweats
○	○	○	○	○	27. Change in appetite	○	○	○	○	○	57. Disorientation or confusion
○	○	○	○	○	28. Restlessness	○	○	○	○	○	58. Flushing (chest, neck and face)
○	○	○	○	○	29. Headache	○	○	○	○	○	59. General aches and pains (joint, muscle, etc.)
○	○	○	○	○	30. Crying	○	○	○	○	○	60. Reduced sense of well-being

Did you menstruate or experience break-through bleeding today? Yes No (circle one)

Did you take any medications today? Yes No (circle one)

Subject No. __________ Today's Date ____ / ____ / ____ Time Completed ____ : ____

Please darken the appropriate circle next to *each* symptom listed below to describe your experience during the past 24 hours (<u>today</u>). Items in **bold print** (#s 7,13,16,23,43 and 52) may be completed immediately upon awakening in the morning; all other items should be completed immediately prior to bedtime. You may use either a pen or pencil.

Respond to each symptom as: (<u>N</u>)ot present; (<u>P</u>)resent, mild; (<u>M</u>)oderate; (<u>S</u>)trong; or, (<u>A</u>)cute, partially disabling.

N	P	M	S	A	
○	○	○	○	○	1. Nausea or vomiting
○	○	○	○	○	2. Loneliness
○	○	○	○	○	3. Hot flashes (upper body warmth/heat)
○	○	○	○	○	4. Anxiety or tension
○	○	○	○	○	5. Numbness or tingling in hands or feet
○	○	○	○	○	6. Cramps (uterine or pelvic)
○	○	○	○	○	**7. Insomnia (difficulty getting to sleep)**
○	○	○	○	○	8. Difficulty concentrating (distractible)
○	○	○	○	○	9. Shortness of breath
○	○	○	○	○	10. Dizziness or faintness
○	○	○	○	○	11. Depression (feeling sad or blue)
○	○	○	○	○	12. Digestive problems
○	○	○	○	○	**13. Night sweats**
○	○	○	○	○	14. Feelings of fear or panic
○	○	○	○	○	15. Chest pains
○	○	○	○	○	**16. Night-time awakenings**
○	○	○	○	○	17. Memory problems (forgetfulness)
○	○	○	○	○	18. Skin tingling/crawling or feels prickly
○	○	○	○	○	19. Loss of interest in usual activities
○	○	○	○	○	20. Clumsiness or increased accidents
○	○	○	○	○	21. Palpitations (heart pounding)
○	○	○	○	○	22. Muscle stiffness
○	○	○	○	○	**23. Early-morning awakenings**
○	○	○	○	○	24. Poor judgment
○	○	○	○	○	25. Backache
○	○	○	○	○	26. Reduced sense of enjoyment
○	○	○	○	○	27. Change in appetite
○	○	○	○	○	28. Restlessness
○	○	○	○	○	29. Headache
○	○	○	○	○	30. Crying

N	P	M	S	A	
○	○	○	○	○	31. Daytime sleepiness
○	○	○	○	○	32. Weight gain
○	○	○	○	○	33. Painful or tender breasts
○	○	○	○	○	34. Feelings of anger or rage
○	○	○	○	○	35. Difficulty making decisions/solving problems
○	○	○	○	○	36. Bursts of energy or activity
○	○	○	○	○	37. Loss of confidence or self-esteem
○	○	○	○	○	38. Worrying
○	○	○	○	○	39. Swelling (breasts, abdomen, ankles, etc.)
○	○	○	○	○	40. Tiredness or fatigue
○	○	○	○	○	41. Increased need for order (orderliness)
○	○	○	○	○	42. Mood swings
○	○	○	○	○	**43. Poor sleep quality**
○	○	○	○	○	44. Frequent urination
○	○	○	○	○	45. Decreased efficiency or lowered performance
○	○	○	○	○	46. Cold shivers
○	○	○	○	○	47. Poor motor coordination
○	○	○	○	○	48. Avoiding social activities
○	○	○	○	○	49. Cravings for specific foods
○	○	○	○	○	50. Irritability
○	○	○	○	○	51. Feelings of suffocation
○	○	○	○	○	**52. Nightmares**
○	○	○	○	○	53. Vision or hearing problems
○	○	○	○	○	54. Feelings of frustration
○	○	○	○	○	55. Hopelessness
○	○	○	○	○	56. Cold sweats
○	○	○	○	○	57. Disorientation or confusion
○	○	○	○	○	58. Flushing (chest, neck and face)
○	○	○	○	○	59. General aches and pains (joint, muscle, etc.)
○	○	○	○	○	60. Reduced sense of well-being

Did you menstruate or experience break-through bleeding <u>today</u>? Yes No (circle one)

Did you take any medications <u>today</u>? Yes No (circle one)

Subject No. ________ Today's Date ___ / ___ / ___ Time Completed ___ : ___

Please darken the appropriate circle next to *each* symptom listed below to describe your experience during the past 24 hours (today). Items in **bold print** (#s 7,13,16,23,43 and 52) may be completed immediately upon awakening in the morning; all other items should be completed immediately prior to bedtime. You may use either a pen or pencil.

Respond to each symptom as: (N)ot present; (P)resent, mild; (M)oderate; (S)trong; or, (A)cute, partially disabling.

N	P	M	S	A	Symptom	N	P	M	S	A	Symptom
○	○	○	○	○	1. Nausea or vomiting	○	○	○	○	○	31. Daytime sleepiness
○	○	○	○	○	2. Loneliness	○	○	○	○	○	32. Weight gain
○	○	○	○	○	3. Hot flashes (upper body warmth/heat)	○	○	○	○	○	33. Painful or tender breasts
○	○	○	○	○	4. Anxiety or tension	○	○	○	○	○	34. Feelings of anger or rage
○	○	○	○	○	5. Numbness or tingling in hands or feet	○	○	○	○	○	35. Difficulty making decisions/solving problems
○	○	○	○	○	6. Cramps (uterine or pelvic)	○	○	○	○	○	36. Bursts of energy or activity
○	○	○	○	○	**7. Insomnia (difficulty getting to sleep)**	○	○	○	○	○	37. Loss of confidence or self-esteem
○	○	○	○	○	8. Difficulty concentrating (distractible)	○	○	○	○	○	38. Worrying
○	○	○	○	○	9. Shortness of breath	○	○	○	○	○	39. Swelling (breasts, abdomen, ankles, etc.)
○	○	○	○	○	10. Dizziness or faintness	○	○	○	○	○	40. Tiredness or fatigue
○	○	○	○	○	11. Depression (feeling sad or blue)	○	○	○	○	○	41. Increased need for order (orderliness)
○	○	○	○	○	12. Digestive problems	○	○	○	○	○	42. Mood swings
○	○	○	○	○	**13. Night sweats**	○	○	○	○	○	**43. Poor sleep quality**
○	○	○	○	○	14. Feelings of fear or panic	○	○	○	○	○	44. Frequent urination
○	○	○	○	○	15. Chest pains	○	○	○	○	○	45. Decreased efficiency or lowered performance
○	○	○	○	○	**16. Night-time awakenings**	○	○	○	○	○	46. Cold shivers
○	○	○	○	○	17. Memory problems (forgetfulness)	○	○	○	○	○	47. Poor motor coordination
○	○	○	○	○	18. Skin tingling/crawling or feels prickly	○	○	○	○	○	48. Avoiding social activities
○	○	○	○	○	19. Loss of interest in usual activities	○	○	○	○	○	49. Cravings for specific foods
○	○	○	○	○	20. Clumsiness or increased accidents	○	○	○	○	○	50. Irritability
○	○	○	○	○	21. Palpitations (heart pounding)	○	○	○	○	○	51. Feelings of suffocation
○	○	○	○	○	22. Muscle stiffness	○	○	○	○	○	**52. Nightmares**
○	○	○	○	○	**23. Early-morning awakenings**	○	○	○	○	○	53. Vision or hearing problems
○	○	○	○	○	24. Poor judgment	○	○	○	○	○	54. Feelings of frustration
○	○	○	○	○	25. Backache	○	○	○	○	○	55. Hopelessness
○	○	○	○	○	26. Reduced sense of enjoyment	○	○	○	○	○	56. Cold sweats
○	○	○	○	○	27. Change in appetite	○	○	○	○	○	57. Disorientation or confusion
○	○	○	○	○	28. Restlessness	○	○	○	○	○	58. Flushing (chest, neck and face)
○	○	○	○	○	29. Headache	○	○	○	○	○	59. General aches and pains (joint, muscle, etc.)
○	○	○	○	○	30. Crying	○	○	○	○	○	60. Reduced sense of well-being

Did you menstruate or experience break-through bleeding today? Yes No (circle one)

Did you take any medications today? Yes No (circle one)

Subject No. __________ Today's Date ____ / ____ / ____ Time Completed ____ : ____

Please darken the appropriate circle next to *each* symptom listed below to describe your experience during the past 24 hours (<u>today</u>). Items in **bold print** (#s 7,13,16,23,43 and 52) may be completed immediately upon awakening in the morning; all other items should be completed immediately prior to bedtime. You may use either a pen or pencil.

Respond to each symptom as: (<u>N</u>)ot present; (<u>P</u>)resent, mild; (<u>M</u>)oderate; (<u>S</u>)trong; or, (<u>A</u>)cute, partially disabling.

N	P	M	S	A	Symptom
○	○	○	○	○	1. Nausea or vomiting
○	○	○	○	○	2. Loneliness
○	○	○	○	○	3. Hot flashes (upper body warmth/heat)
○	○	○	○	○	4. Anxiety or tension
○	○	○	○	○	5. Numbness or tingling in hands or feet
○	○	○	○	○	6. Cramps (uterine or pelvic)
○	○	○	○	○	**7. Insomnia (difficulty getting to sleep)**
○	○	○	○	○	8. Difficulty concentrating (distractible)
○	○	○	○	○	9. Shortness of breath
○	○	○	○	○	10. Dizziness or faintness
○	○	○	○	○	11. Depression (feeling sad or blue)
○	○	○	○	○	12. Digestive problems
○	○	○	○	○	**13. Night sweats**
○	○	○	○	○	14. Feelings of fear or panic
○	○	○	○	○	15. Chest pains
○	○	○	○	○	**16. Night-time awakenings**
○	○	○	○	○	17. Memory problems (forgetfulness)
○	○	○	○	○	18. Skin tingling/crawling or feels prickly
○	○	○	○	○	19. Loss of interest in usual activities
○	○	○	○	○	20. Clumsiness or increased accidents
○	○	○	○	○	21. Palpitations (heart pounding)
○	○	○	○	○	22. Muscle stiffness
○	○	○	○	○	**23. Early-morning awakenings**
○	○	○	○	○	24. Poor judgment
○	○	○	○	○	25. Backache
○	○	○	○	○	26. Reduced sense of enjoyment
○	○	○	○	○	27. Change in appetite
○	○	○	○	○	28. Restlessness
○	○	○	○	○	29. Headache
○	○	○	○	○	30. Crying

N	P	M	S	A	Symptom
○	○	○	○	○	31. Daytime sleepiness
○	○	○	○	○	32. Weight gain
○	○	○	○	○	33. Painful or tender breasts
○	○	○	○	○	34. Feelings of anger or rage
○	○	○	○	○	35. Difficulty making decisions/solving problems
○	○	○	○	○	36. Bursts of energy or activity
○	○	○	○	○	37. Loss of confidence or self-esteem
○	○	○	○	○	38. Worrying
○	○	○	○	○	39. Swelling (breasts, abdomen, ankles, etc.)
○	○	○	○	○	40. Tiredness or fatigue
○	○	○	○	○	41. Increased need for order (orderliness)
○	○	○	○	○	42. Mood swings
○	○	○	○	○	**43. Poor sleep quality**
○	○	○	○	○	44. Frequent urination
○	○	○	○	○	45. Decreased efficiency or lowered performance
○	○	○	○	○	46. Cold shivers
○	○	○	○	○	47. Poor motor coordination
○	○	○	○	○	48. Avoiding social activities
○	○	○	○	○	49. Cravings for specific foods
○	○	○	○	○	50. Irritability
○	○	○	○	○	51. Feelings of suffocation
○	○	○	○	○	**52. Nightmares**
○	○	○	○	○	53. Vision or hearing problems
○	○	○	○	○	54. Feelings of frustration
○	○	○	○	○	55. Hopelessness
○	○	○	○	○	56. Cold sweats
○	○	○	○	○	57. Disorientation or confusion
○	○	○	○	○	58. Flushing (chest, neck and face)
○	○	○	○	○	59. General aches and pains (joint, muscle, etc.)
○	○	○	○	○	60. Reduced sense of well-being

Did you menstruate or experience break-through bleeding <u>today</u>? Yes No (circle one)

Did you take any medications <u>today</u>? Yes No (circle one)

Subject No. __________ Today's Date ____ / ____ / ____ Time Completed ____ : ____

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N	P	M	S	A	Symptom	N	P	M	S	A	Symptom
○	○	○	○	○	1. Nausea or vomiting	○	○	○	○	○	31. Daytime sleepiness
○	○	○	○	○	2. Loneliness	○	○	○	○	○	32. Weight gain
○	○	○	○	○	3. Hot flashes (upper body warmth/heat)	○	○	○	○	○	33. Painful or tender breasts
○	○	○	○	○	4. Anxiety or tension	○	○	○	○	○	34. Feelings of anger or rage
○	○	○	○	○	5. Numbness or tingling in hands or feet	○	○	○	○	○	35. Difficulty making decisions/solving problems
○	○	○	○	○	6. Cramps (uterine or pelvic)	○	○	○	○	○	36. Bursts of energy or activity
○	○	○	○	○	**7. Insomnia (difficulty getting to sleep)**	○	○	○	○	○	37. Loss of confidence or self-esteem
○	○	○	○	○	8. Difficulty concentrating (distractible)	○	○	○	○	○	38. Worrying
○	○	○	○	○	9. Shortness of breath	○	○	○	○	○	39. Swelling (breasts, abdomen, ankles, etc.)
○	○	○	○	○	10. Dizziness or faintness	○	○	○	○	○	40. Tiredness or fatigue
○	○	○	○	○	11. Depression (feeling sad or blue)	○	○	○	○	○	41. Increased need for order (orderliness)
○	○	○	○	○	12. Digestive problems	○	○	○	○	○	42. Mood swings
○	○	○	○	○	**13. Night sweats**	○	○	○	○	○	**43. Poor sleep quality**
○	○	○	○	○	14. Feelings of fear or panic	○	○	○	○	○	44. Frequent urination
○	○	○	○	○	15. Chest pains	○	○	○	○	○	45. Decreased efficiency or lowered performance
○	○	○	○	○	**16. Night-time awakenings**	○	○	○	○	○	46. Cold shivers
○	○	○	○	○	17. Memory problems (forgetfulness)	○	○	○	○	○	47. Poor motor coordination
○	○	○	○	○	18. Skin tingling/crawling or feels prickly	○	○	○	○	○	48. Avoiding social activities
○	○	○	○	○	19. Loss of interest in usual activities	○	○	○	○	○	49. Cravings for specific foods
○	○	○	○	○	20. Clumsiness or increased accidents	○	○	○	○	○	50. Irritability
○	○	○	○	○	21. Palpitations (heart pounding)	○	○	○	○	○	51. Feelings of suffocation
○	○	○	○	○	22. Muscle stiffness	○	○	○	○	○	**52. Nightmares**
○	○	○	○	○	**23. Early-morning awakenings**	○	○	○	○	○	53. Vision or hearing problems
○	○	○	○	○	24. Poor judgment	○	○	○	○	○	54. Feelings of frustration
○	○	○	○	○	25. Backache	○	○	○	○	○	55. Hopelessness
○	○	○	○	○	26. Reduced sense of enjoyment	○	○	○	○	○	56. Cold sweats
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○	○	○	○	○	28. Restlessness	○	○	○	○	○	58. Flushing (chest, neck and face)
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○	○	○	○	○	30. Crying	○	○	○	○	○	60. Reduced sense of well-being

Did you menstruate or experience break-through bleeding today? Yes No (circle one)

Did you take any medications today? Yes No (circle one)

Subject No. __________ Today's Date ____ / ____ / ____ Time Completed ____ : ____

Please darken the appropriate circle next to *each* symptom listed below to describe your experience during the past 24 hours (today). Items in **bold print** (#s 7,13,16,23,43 and 52) may be completed immediately upon awakening in the morning; all other items should be completed immediately prior to bedtime. You may use either a pen or pencil.

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N	P	M	S	A	Symptom
○	○	○	○	○	1. Nausea or vomiting
○	○	○	○	○	2. Loneliness
○	○	○	○	○	3. Hot flashes (upper body warmth/heat)
○	○	○	○	○	4. Anxiety or tension
○	○	○	○	○	5. Numbness or tingling in hands or feet
○	○	○	○	○	6. Cramps (uterine or pelvic)
○	○	○	○	○	**7. Insomnia (difficulty getting to sleep)**
○	○	○	○	○	8. Difficulty concentrating (distractible)
○	○	○	○	○	9. Shortness of breath
○	○	○	○	○	10. Dizziness or faintness
○	○	○	○	○	11. Depression (feeling sad or blue)
○	○	○	○	○	12. Digestive problems
○	○	○	○	○	**13. Night sweats**
○	○	○	○	○	14. Feelings of fear or panic
○	○	○	○	○	15. Chest pains
○	○	○	○	○	**16. Night-time awakenings**
○	○	○	○	○	17. Memory problems (forgetfulness)
○	○	○	○	○	18. Skin tingling/crawling or feels prickly
○	○	○	○	○	19. Loss of interest in usual activities
○	○	○	○	○	20. Clumsiness or increased accidents
○	○	○	○	○	21. Palpitations (heart pounding)
○	○	○	○	○	22. Muscle stiffness
○	○	○	○	○	**23. Early-morning awakenings**
○	○	○	○	○	24. Poor judgment
○	○	○	○	○	25. Backache
○	○	○	○	○	26. Reduced sense of enjoyment
○	○	○	○	○	27. Change in appetite
○	○	○	○	○	28. Restlessness
○	○	○	○	○	29. Headache
○	○	○	○	○	30. Crying

N	P	M	S	A	Symptom
○	○	○	○	○	31. Daytime sleepiness
○	○	○	○	○	32. Weight gain
○	○	○	○	○	33. Painful or tender breasts
○	○	○	○	○	34. Feelings of anger or rage
○	○	○	○	○	35. Difficulty making decisions/solving problems
○	○	○	○	○	36. Bursts of energy or activity
○	○	○	○	○	37. Loss of confidence or self-esteem
○	○	○	○	○	38. Worrying
○	○	○	○	○	39. Swelling (breasts, abdomen, ankles, etc.)
○	○	○	○	○	40. Tiredness or fatigue
○	○	○	○	○	41. Increased need for order (orderliness)
○	○	○	○	○	42. Mood swings
○	○	○	○	○	**43. Poor sleep quality**
○	○	○	○	○	44. Frequent urination
○	○	○	○	○	45. Decreased efficiency or lowered performance
○	○	○	○	○	46. Cold shivers
○	○	○	○	○	47. Poor motor coordination
○	○	○	○	○	48. Avoiding social activities
○	○	○	○	○	49. Cravings for specific foods
○	○	○	○	○	50. Irritability
○	○	○	○	○	51. Feelings of suffocation
○	○	○	○	○	**52. Nightmares**
○	○	○	○	○	53. Vision or hearing problems
○	○	○	○	○	54. Feelings of frustration
○	○	○	○	○	55. Hopelessness
○	○	○	○	○	56. Cold sweats
○	○	○	○	○	57. Disorientation or confusion
○	○	○	○	○	58. Flushing (chest, neck and face)
○	○	○	○	○	59. General aches and pains (joint, muscle, etc.)
○	○	○	○	○	60. Reduced sense of well-being

Did you menstruate or experience break-through bleeding today? Yes No (circle one)

Did you take any medications today? Yes No (circle one)

Subject No. __________ Today's Date ____ / ____ / ____ Time Completed ____ : ____

Please darken the appropriate circle next to *each* symptom listed below to describe your experience during the past 24 hours (today). Items in **bold print** (#s 7,13,16,23,43 and 52) may be completed immediately upon awakening in the morning; all other items should be completed immediately prior to bedtime. You may use either a pen or pencil.

Respond to each symptom as: (N)ot present; (P)resent, mild; (M)oderate; (S)trong; or, (A)cute, partially disabling.

N	P	M	S	A	
○	○	○	○	○	1. Nausea or vomiting
○	○	○	○	○	2. Loneliness
○	○	○	○	○	3. Hot flashes (upper body warmth/heat)
○	○	○	○	○	4. Anxiety or tension
○	○	○	○	○	5. Numbness or tingling in hands or feet
○	○	○	○	○	6. Cramps (uterine or pelvic)
○	○	○	○	○	**7. Insomnia (difficulty getting to sleep)**
○	○	○	○	○	8. Difficulty concentrating (distractible)
○	○	○	○	○	9. Shortness of breath
○	○	○	○	○	10. Dizziness or faintness
○	○	○	○	○	11. Depression (feeling sad or blue)
○	○	○	○	○	12. Digestive problems
○	○	○	○	○	**13. Night sweats**
○	○	○	○	○	14. Feelings of fear or panic
○	○	○	○	○	15. Chest pains
○	○	○	○	○	**16. Night-time awakenings**
○	○	○	○	○	17. Memory problems (forgetfulness)
○	○	○	○	○	18. Skin tingling/crawling or feels prickly
○	○	○	○	○	19. Loss of interest in usual activities
○	○	○	○	○	20. Clumsiness or increased accidents
○	○	○	○	○	21. Palpitations (heart pounding)
○	○	○	○	○	22. Muscle stiffness
○	○	○	○	○	**23. Early-morning awakenings**
○	○	○	○	○	24. Poor judgment
○	○	○	○	○	25. Backache
○	○	○	○	○	26. Reduced sense of enjoyment
○	○	○	○	○	27. Change in appetite
○	○	○	○	○	28. Restlessness
○	○	○	○	○	29. Headache
○	○	○	○	○	30. Crying

N	P	M	S	A	
○	○	○	○	○	31. Daytime sleepiness
○	○	○	○	○	32. Weight gain
○	○	○	○	○	33. Painful or tender breasts
○	○	○	○	○	34. Feelings of anger or rage
○	○	○	○	○	35. Difficulty making decisions/solving problems
○	○	○	○	○	36. Bursts of energy or activity
○	○	○	○	○	37. Loss of confidence or self-esteem
○	○	○	○	○	38. Worrying
○	○	○	○	○	39. Swelling (breasts, abdomen, ankles, etc.)
○	○	○	○	○	40. Tiredness or fatigue
○	○	○	○	○	41. Increased need for order (orderliness)
○	○	○	○	○	42. Mood swings
○	○	○	○	○	**43. Poor sleep quality**
○	○	○	○	○	44. Frequent urination
○	○	○	○	○	45. Decreased efficiency or lowered performance
○	○	○	○	○	46. Cold shivers
○	○	○	○	○	47. Poor motor coordination
○	○	○	○	○	48. Avoiding social activities
○	○	○	○	○	49. Cravings for specific foods
○	○	○	○	○	50. Irritability
○	○	○	○	○	51. Feelings of suffocation
○	○	○	○	○	**52. Nightmares**
○	○	○	○	○	53. Vision or hearing problems
○	○	○	○	○	54. Feelings of frustration
○	○	○	○	○	55. Hopelessness
○	○	○	○	○	56. Cold sweats
○	○	○	○	○	57. Disorientation or confusion
○	○	○	○	○	58. Flushing (chest, neck and face)
○	○	○	○	○	59. General aches and pains (joint, muscle, etc.)
○	○	○	○	○	60. Reduced sense of well-being

Did you menstruate or experience break-through bleeding today? Yes No (circle one)

Did you take any medications today? Yes No (circle one)

1/94 USDA/ARS/NPA/GFHNRC/PRG/JGP MDQ-ST

Subject No. __________ Today's Date ____ / ____ / ____ Time Completed ____ : ____

Please darken the appropriate circle next to *each* symptom listed below to describe your experience during the past 24 hours (today). Items in **bold print** (#s 7,13,16,23,43 and 52) may be completed immediately upon awakening in the morning; all other items should be completed immediately prior to bedtime. You may use either a pen or pencil.

Respond to each symptom as: (N)ot present; (P)resent, mild; (M)oderate; (S)trong; or, (A)cute, partially disabling.

N	P	M	S	A	Symptom
○	○	○	○	○	1. Nausea or vomiting
○	○	○	○	○	2. Loneliness
○	○	○	○	○	3. Hot flashes (upper body warmth/heat)
○	○	○	○	○	4. Anxiety or tension
○	○	○	○	○	5. Numbness or tingling in hands or feet
○	○	○	○	○	6. Cramps (uterine or pelvic)
○	○	○	○	○	**7. Insomnia (difficulty getting to sleep)**
○	○	○	○	○	8. Difficulty concentrating (distractible)
○	○	○	○	○	9. Shortness of breath
○	○	○	○	○	10. Dizziness or faintness
○	○	○	○	○	11. Depression (feeling sad or blue)
○	○	○	○	○	12. Digestive problems
○	○	○	○	○	**13. Night sweats**
○	○	○	○	○	14. Feelings of fear or panic
○	○	○	○	○	15. Chest pains
○	○	○	○	○	**16. Night-time awakenings**
○	○	○	○	○	17. Memory problems (forgetfulness)
○	○	○	○	○	18. Skin tingling/crawling or feels prickly
○	○	○	○	○	19. Loss of interest in usual activities
○	○	○	○	○	20. Clumsiness or increased accidents
○	○	○	○	○	21. Palpitations (heart pounding)
○	○	○	○	○	22. Muscle stiffness
○	○	○	○	○	**23. Early-morning awakenings**
○	○	○	○	○	24. Poor judgment
○	○	○	○	○	25. Backache
○	○	○	○	○	26. Reduced sense of enjoyment
○	○	○	○	○	27. Change in appetite
○	○	○	○	○	28. Restlessness
○	○	○	○	○	29. Headache
○	○	○	○	○	30. Crying

N	P	M	S	A	Symptom
○	○	○	○	○	31. Daytime sleepiness
○	○	○	○	○	32. Weight gain
○	○	○	○	○	33. Painful or tender breasts
○	○	○	○	○	34. Feelings of anger or rage
○	○	○	○	○	35. Difficulty making decisions/solving problems
○	○	○	○	○	36. Bursts of energy or activity
○	○	○	○	○	37. Loss of confidence or self-esteem
○	○	○	○	○	38. Worrying
○	○	○	○	○	39. Swelling (breasts, abdomen, ankles, etc.)
○	○	○	○	○	40. Tiredness or fatigue
○	○	○	○	○	41. Increased need for order (orderliness)
○	○	○	○	○	42. Mood swings
○	○	○	○	○	**43. Poor sleep quality**
○	○	○	○	○	44. Frequent urination
○	○	○	○	○	45. Decreased efficiency or lowered performance
○	○	○	○	○	46. Cold shivers
○	○	○	○	○	47. Poor motor coordination
○	○	○	○	○	48. Avoiding social activities
○	○	○	○	○	49. Cravings for specific foods
○	○	○	○	○	50. Irritability
○	○	○	○	○	51. Feelings of suffocation
○	○	○	○	○	**52. Nightmares**
○	○	○	○	○	53. Vision or hearing problems
○	○	○	○	○	54. Feelings of frustration
○	○	○	○	○	55. Hopelessness
○	○	○	○	○	56. Cold sweats
○	○	○	○	○	57. Disorientation or confusion
○	○	○	○	○	58. Flushing (chest, neck and face)
○	○	○	○	○	59. General aches and pains (joint, muscle, etc.)
○	○	○	○	○	60. Reduced sense of well-being

Did you menstruate or experience break-through bleeding today? Yes No (circle one)

Did you take any medications today? Yes No (circle one)

Subject No. __________ Today's Date ____ / ____ / ____ Time Completed ____ : ____

Please darken the appropriate circle next to *each* symptom listed below to describe your experience during the past 24 hours (today). Items in **bold print** (#s 7,13,16,23,43 and 52) may be completed immediately upon awakening in the morning; all other items should be completed immediately prior to bedtime. You may use either a pen or pencil.

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N	P	M	S	A	Symptom
○	○	○	○	○	1. Nausea or vomiting
○	○	○	○	○	2. Loneliness
○	○	○	○	○	3. Hot flashes (upper body warmth/heat)
○	○	○	○	○	4. Anxiety or tension
○	○	○	○	○	5. Numbness or tingling in hands or feet
○	○	○	○	○	6. Cramps (uterine or pelvic)
○	○	○	○	○	**7. Insomnia (difficulty getting to sleep)**
○	○	○	○	○	8. Difficulty concentrating (distractible)
○	○	○	○	○	9. Shortness of breath
○	○	○	○	○	10. Dizziness or faintness
○	○	○	○	○	11. Depression (feeling sad or blue)
○	○	○	○	○	12. Digestive problems
○	○	○	○	○	**13. Night sweats**
○	○	○	○	○	14. Feelings of fear or panic
○	○	○	○	○	15. Chest pains
○	○	○	○	○	**16. Night-time awakenings**
○	○	○	○	○	17. Memory problems (forgetfulness)
○	○	○	○	○	18. Skin tingling/crawling or feels prickly
○	○	○	○	○	19. Loss of interest in usual activities
○	○	○	○	○	20. Clumsiness or increased accidents
○	○	○	○	○	21. Palpitations (heart pounding)
○	○	○	○	○	22. Muscle stiffness
○	○	○	○	○	**23. Early-morning awakenings**
○	○	○	○	○	24. Poor judgment
○	○	○	○	○	25. Backache
○	○	○	○	○	26. Reduced sense of enjoyment
○	○	○	○	○	27. Change in appetite
○	○	○	○	○	28. Restlessness
○	○	○	○	○	29. Headache
○	○	○	○	○	30. Crying

N	P	M	S	A	Symptom
○	○	○	○	○	31. Daytime sleepiness
○	○	○	○	○	32. Weight gain
○	○	○	○	○	33. Painful or tender breasts
○	○	○	○	○	34. Feelings of anger or rage
○	○	○	○	○	35. Difficulty making decisions/solving problems
○	○	○	○	○	36. Bursts of energy or activity
○	○	○	○	○	37. Loss of confidence or self-esteem
○	○	○	○	○	38. Worrying
○	○	○	○	○	39. Swelling (breasts, abdomen, ankles, etc.)
○	○	○	○	○	40. Tiredness or fatigue
○	○	○	○	○	41. Increased need for order (orderliness)
○	○	○	○	○	42. Mood swings
○	○	○	○	○	**43. Poor sleep quality**
○	○	○	○	○	44. Frequent urination
○	○	○	○	○	45. Decreased efficiency or lowered performance
○	○	○	○	○	46. Cold shivers
○	○	○	○	○	47. Poor motor coordination
○	○	○	○	○	48. Avoiding social activities
○	○	○	○	○	49. Cravings for specific foods
○	○	○	○	○	50. Irritability
○	○	○	○	○	51. Feelings of suffocation
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○	○	○	○	○	53. Vision or hearing problems
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○	○	○	○	○	55. Hopelessness
○	○	○	○	○	56. Cold sweats
○	○	○	○	○	57. Disorientation or confusion
○	○	○	○	○	58. Flushing (chest, neck and face)
○	○	○	○	○	59. General aches and pains (joint, muscle, etc.)
○	○	○	○	○	60. Reduced sense of well-being

Did you menstruate or experience break-through bleeding today? Yes No (circle one)

Did you take any medications today? Yes No (circle one)

Subject No. ________ Today's Date ___ / ___ / ___ Time Completed ___ : ___

Please darken the appropriate circle next to *each* symptom listed below to describe your experience during the past 24 hours (today). Items in **bold print** (#s 7,13,16,23,43 and 52) may be completed immediately upon awakening in the morning; all other items should be completed immediately prior to bedtime. You may use either a pen or pencil.

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N	P	M	S	A	Symptom
○	○	○	○	○	1. Nausea or vomiting
○	○	○	○	○	2. Loneliness
○	○	○	○	○	3. Hot flashes (upper body warmth/heat)
○	○	○	○	○	4. Anxiety or tension
○	○	○	○	○	5. Numbness or tingling in hands or feet
○	○	○	○	○	6. Cramps (uterine or pelvic)
○	○	○	○	○	**7. Insomnia (difficulty getting to sleep)**
○	○	○	○	○	8. Difficulty concentrating (distractible)
○	○	○	○	○	9. Shortness of breath
○	○	○	○	○	10. Dizziness or faintness
○	○	○	○	○	11. Depression (feeling sad or blue)
○	○	○	○	○	12. Digestive problems
○	○	○	○	○	**13. Night sweats**
○	○	○	○	○	14. Feelings of fear or panic
○	○	○	○	○	15. Chest pains
○	○	○	○	○	**16. Night-time awakenings**
○	○	○	○	○	17. Memory problems (forgetfulness)
○	○	○	○	○	18. Skin tingling/crawling or feels prickly
○	○	○	○	○	19. Loss of interest in usual activities
○	○	○	○	○	20. Clumsiness or increased accidents
○	○	○	○	○	21. Palpitations (heart pounding)
○	○	○	○	○	22. Muscle stiffness
○	○	○	○	○	**23. Early-morning awakenings**
○	○	○	○	○	24. Poor judgment
○	○	○	○	○	25. Backache
○	○	○	○	○	26. Reduced sense of enjoyment
○	○	○	○	○	27. Change in appetite
○	○	○	○	○	28. Restlessness
○	○	○	○	○	29. Headache
○	○	○	○	○	30. Crying

N	P	M	S	A	Symptom
○	○	○	○	○	31. Daytime sleepiness
○	○	○	○	○	32. Weight gain
○	○	○	○	○	33. Painful or tender breasts
○	○	○	○	○	34. Feelings of anger or rage
○	○	○	○	○	35. Difficulty making decisions/solving problems
○	○	○	○	○	36. Bursts of energy or activity
○	○	○	○	○	37. Loss of confidence or self-esteem
○	○	○	○	○	38. Worrying
○	○	○	○	○	39. Swelling (breasts, abdomen, ankles, etc.)
○	○	○	○	○	40. Tiredness or fatigue
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○	○	○	○	○	48. Avoiding social activities
○	○	○	○	○	49. Cravings for specific foods
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○	○	○	○	○	51. Feelings of suffocation
○	○	○	○	○	**52. Nightmares**
○	○	○	○	○	53. Vision or hearing problems
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○	○	○	○	○	56. Cold sweats
○	○	○	○	○	57. Disorientation or confusion
○	○	○	○	○	58. Flushing (chest, neck and face)
○	○	○	○	○	59. General aches and pains (joint, muscle, etc.)
○	○	○	○	○	60. Reduced sense of well-being

Did you menstruate or experience break-through bleeding today? Yes No (circle one)

Did you take any medications today? Yes No (circle one)

Subject No. __________ Today's Date ____ / ____ / ____ Time Completed ____ : ____

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N	P	M	S	A	Symptom	N	P	M	S	A	Symptom
○	○	○	○	○	1. Nausea or vomiting	○	○	○	○	○	31. Daytime sleepiness
○	○	○	○	○	2. Loneliness	○	○	○	○	○	32. Weight gain
○	○	○	○	○	3. Hot flashes (upper body warmth/heat)	○	○	○	○	○	33. Painful or tender breasts
○	○	○	○	○	4. Anxiety or tension	○	○	○	○	○	34. Feelings of anger or rage
○	○	○	○	○	5. Numbness or tingling in hands or feet	○	○	○	○	○	35. Difficulty making decisions/solving problems
○	○	○	○	○	6. Cramps (uterine or pelvic)	○	○	○	○	○	36. Bursts of energy or activity
○	○	○	○	○	**7. Insomnia (difficulty getting to sleep)**	○	○	○	○	○	37. Loss of confidence or self-esteem
○	○	○	○	○	8. Difficulty concentrating (distractible)	○	○	○	○	○	38. Worrying
○	○	○	○	○	9. Shortness of breath	○	○	○	○	○	39. Swelling (breasts, abdomen, ankles, etc.)
○	○	○	○	○	10. Dizziness or faintness	○	○	○	○	○	40. Tiredness or fatigue
○	○	○	○	○	11. Depression (feeling sad or blue)	○	○	○	○	○	41. Increased need for order (orderliness)
○	○	○	○	○	12. Digestive problems	○	○	○	○	○	42. Mood swings
○	○	○	○	○	**13. Night sweats**	○	○	○	○	○	**43. Poor sleep quality**
○	○	○	○	○	14. Feelings of fear or panic	○	○	○	○	○	44. Frequent urination
○	○	○	○	○	15. Chest pains	○	○	○	○	○	45. Decreased efficiency or lowered performance
○	○	○	○	○	**16. Night-time awakenings**	○	○	○	○	○	46. Cold shivers
○	○	○	○	○	17. Memory problems (forgetfulness)	○	○	○	○	○	47. Poor motor coordination
○	○	○	○	○	18. Skin tingling/crawling or feels prickly	○	○	○	○	○	48. Avoiding social activities
○	○	○	○	○	19. Loss of interest in usual activities	○	○	○	○	○	49. Cravings for specific foods
○	○	○	○	○	20. Clumsiness or increased accidents	○	○	○	○	○	50. Irritability
○	○	○	○	○	21. Palpitations (heart pounding)	○	○	○	○	○	51. Feelings of suffocation
○	○	○	○	○	22. Muscle stiffness	○	○	○	○	○	**52. Nightmares**
○	○	○	○	○	**23. Early-morning awakenings**	○	○	○	○	○	53. Vision or hearing problems
○	○	○	○	○	24. Poor judgment	○	○	○	○	○	54. Feelings of frustration
○	○	○	○	○	25. Backache	○	○	○	○	○	55. Hopelessness
○	○	○	○	○	26. Reduced sense of enjoyment	○	○	○	○	○	56. Cold sweats
○	○	○	○	○	27. Change in appetite	○	○	○	○	○	57. Disorientation or confusion
○	○	○	○	○	28. Restlessness	○	○	○	○	○	58. Flushing (chest, neck and face)
○	○	○	○	○	29. Headache	○	○	○	○	○	59. General aches and pains (joint, muscle, etc.)
○	○	○	○	○	30. Crying	○	○	○	○	○	60. Reduced sense of well-being

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○	○	○	○	○	2. Loneliness	○	○	○	○	○	32. Weight gain
○	○	○	○	○	3. Hot flashes (upper body warmth/heat)	○	○	○	○	○	33. Painful or tender breasts
○	○	○	○	○	4. Anxiety or tension	○	○	○	○	○	34. Feelings of anger or rage
○	○	○	○	○	5. Numbness or tingling in hands or feet	○	○	○	○	○	35. Difficulty making decisions/solving problems
○	○	○	○	○	6. Cramps (uterine or pelvic)	○	○	○	○	○	36. Bursts of energy or activity
○	○	○	○	○	**7. Insomnia (difficulty getting to sleep)**	○	○	○	○	○	37. Loss of confidence or self-esteem
○	○	○	○	○	8. Difficulty concentrating (distractible)	○	○	○	○	○	38. Worrying
○	○	○	○	○	9. Shortness of breath	○	○	○	○	○	39. Swelling (breasts, abdomen, ankles, etc.)
○	○	○	○	○	10. Dizziness or faintness	○	○	○	○	○	40. Tiredness or fatigue
○	○	○	○	○	11. Depression (feeling sad or blue)	○	○	○	○	○	41. Increased need for order (orderliness)
○	○	○	○	○	12. Digestive problems	○	○	○	○	○	42. Mood swings
○	○	○	○	○	**13. Night sweats**	○	○	○	○	○	**43. Poor sleep quality**
○	○	○	○	○	14. Feelings of fear or panic	○	○	○	○	○	44. Frequent urination
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○	○	○	○	○	5. Numbness or tingling in hands or feet
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Comparison of Tri-Exponential and Truncated Models in Plasam Zinc (Zn) Kinetics.

K Yokoi, VM Sadagopa Ramanujam, NG Egger, HH Dayal, NW Alcock and HH Sandstead.

University of Texas Medical Branch, Galveston, TX and Kyoto University Graduate School of Medicine, Kyoto, Japan.

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The Drake Hotel, Chicago, Illinois

Abstract

Current clinical indices for evaluating Zn status are imperfect. Prasad et al (1963) used the plasma Zn disappearance to show that growth stunted adolescents were low in Zn. Miller et al (1994) found that Zn pool size related to dietary Zn using stable Zn isotopes and fast atom bombardment mass spectrometry (FAB MS). We therefore compared kinetic parameters after i.v. ^{67}Zn derived from 0 - 9 days (tri-exponential) and from 0 - 24 hours (truncated) plasma collections in six subjects (5 men and 1 woman, age 24 - 64 y, BMI 23.2 - 30.4). Plasma Zn isotope ratios (IR) were measured by inductively coupled plasma - mass spectrometry (ICP-MS). The experimental IR was corrected for the baseline and divided by the natural Zn IR to give the normalized IR (NIR). Plasma Zn disappearance from 0 - 9 days was well explained ($R^2 = 0.99$) by the tri-exponential model: $NIR = K_1 \exp(-g_1 t) + K_2 \exp(-g_2 t) + K_3 \exp(-g_3 t)$, where t is time in days. NIR from 0 - 24 hours was analyzed using the truncated model: $NIR = K_1 \exp(-g_1 t) + K_2 \exp(-g_2 t) + K_3$, $R^2 = 0.98 - 0.99$. The estimated coefficients from the tri-exponential and truncated models were similar except for g_2. The so-called 'rapidly' exchanging Zn pool (EZP) and related parameters determined from the 0 - 9 day plasma samples using a tri-exponential and an open mammillary model are predictable from 0 - 24 hours observation using a truncated and a closed mammillary model. One day plasma Zn pool approximated EZP better than extrapolation of the 3 - 9 day plasma NIR. The truncated model for 0 - 24 hours is therefore concordant with the full tri-exponential model for 0 -9 days.

Introduction

Biochemical indices for evaluating Zn status are imperfect and the specificity of physiological indices are unknown. Zn kinetic parameters have been measured using radioactive Zn. Prasad et al (1963) used ^{65}Zn to show rapid disappearance of plasma Zn in growth stunted adolescents. Using ^{69m}Zn and ^{65}Zn Aamodt et al (1979, 1982) observed the change of Zn kinetics after oral loading of 100 mg Zn. Foster et al (1984) and Wastney et al (1986) developed an integrated Zn kinetic model using ^{65}Zn and ^{69m}Zn.

Stable Zn isotopes are alternatives. Wastney et al (1991) compared the results obtained from ^{65}Zn and ^{70}Zn using neutron activation analysis. Miller et al (1991, 1994) used stable Zn isotopes and FABMS for measuring Zn pools. Using Quadrapole ICP-MS, Lowe et al (1992) analyzed the 120 min kinetics with ^{70}Zn and Yokoi et al (1992, 1994a, 1994b) evaluated Zn disappearance from 30 to 60 min after iv dose of ^{67}Zn. Fairweather-Tait et al (1993) measured Zn pools using ^{70}Zn and thermal ionization mass spectrometry (TIMS). Scott and Turnlund (1994) adapted Wastney's approach (1986) to ^{67}Zn and ^{70}Zn tracer using TIMS.

There are two mathematical approaches. 1. The deconvolution method (Foster 1979, 1984; Wastney 1986) which treats remaining Zn tracer in plasma as a forcing function in the convolution integral (Berman, 1978). 2. The conventional compartment method based on coefficients in the polyexponential function fitted to the remaining tracer in plasma. The kinetic parameters depend on the observation intervals. We therefore investigated a short-term kinetics concordant with the long-term kinetics using ^{67}Zn and Quadrapole ICP-MS. To our knowledge, this truncated model approach has never been applied to determine Zn kinetic parameters.

Objective

- To develop a method for determination of plasma zinc kinetic parameters, based on data obtained from a short observation period (within 1 day, truncated model)
- To compare the results of the truncated model and those obtained from a long term observation period (0 - 9 days)

Method

1. Inject 1.78 mg ^{67}Zn intravenously to human subjects and collect blood samples at baseline (before the administration) and 5, 15, 30, 40, 50, 60, 90 minutes, 2, 6, 12 hours, 1, (2), 3, 5, 7, 9 days later.

2. Digest the specimens, extract Zn using diethylammonium diethyldithiocarbamate (chelator) and carbon tetrachloride (organic solvent) and measure the Zn isotope ratio (^{67}Zn:^{64}Zn, ^{67}Zn:^{66}Zn, ^{67}Zn:^{68}Zn, ^{67}Zn:^{70}Zn) by ICP-MS (Plasma Quad, VG Instruments) based on Yokoi et al (1994a; 1994b).

3. Subtract the baseline from the measured isotope ratio and divide the ratio by natural Zn isotope ratio to obtain the normalized isotope ratio (NIR).

4. Analyze NIR data from 5 min to 1 day with the truncated model; NIR from 5 min to 9 days with the tri-exponential model using nonlinear regression.

5. Calculate the kinetic parameters using the 'concentric design' or the mammillary model from the obtained coefficients of the polyexponential function.

6. Compare the parameters obtained from various models and observation intervals.

Parameter estimates from various observation periods obtained from our analysis of Wastney et al's data (1986)

Parameter	0 - 290 days	0 - 28 days	0 - 2 days
K_1	**1.18**	**1.18**	**1.17**
g_1	**131**	**131**	**130**
K_2	**0.0433**	**0.0427**	**0.0451**
g_2	**4.50**	**4.66**	**4.19**
K_3	**0.0136**	**0.0143**	**0.0136**
g_3	**0.104**	**0.118**	
K_4	**0.00215**	**0.00228**	
g_4	**0.00439**		

The results indicate the appropriate number of terms in the polyexponential function concordant with the longest observation intervals as follows:

1 *or* 2 *days*: $K_1 e^{-g_1} + K_2 e^{-g_2} + K_3$

9 *days*: $K_1 e^{-g_1} + K_2 e^{-g_2} + K_3 e^{-g_3}$

1 *month*: $K_1 e^{-g_1} + K_2 e^{-g_2} + K_3 e^{-g_3} + K_4$

10 *months*: $K_1 e^{-g_1} + K_2 e^{-g_2} + K_3 e^{-g_3} + K_4 e^{-g_4}$

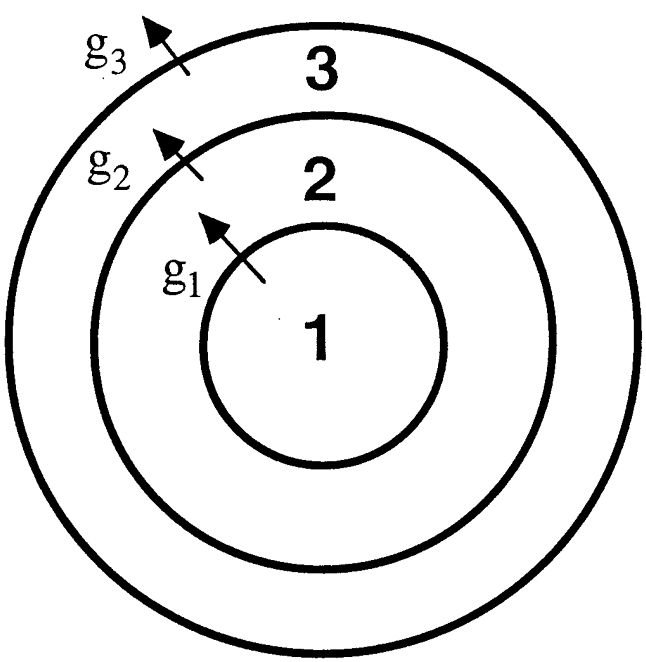
where 'K's are linear coefficients; 'g's are exponential coefficients.

Simple Interpretation of Tri-Exponential with the 'Concentric Design'

If $K_1 >> K_2 >> K_3$ and $g_1 >> g_2 >> g_3$ are satisfied,

Pool 1 = Plasma Zn pool
= Injected tracer (mmol)/ Extrapolated tracer in plasma at t=0
Pool 2 = Plasma Zn pool/$(1-H_1)$ = Plasma Zn pool/(H_2+H_3)
Pool 3 = Plasma Zn pool/$(1-H_1-H_2)$ = Plasma Zn pool/H_3

$H_1 = K_1/(K_1+K_2+K_3)$ $H_2=K_2/(K_1+K_2+K_3)$ $H_3=K_3/(K_1+K_2+K_3)$

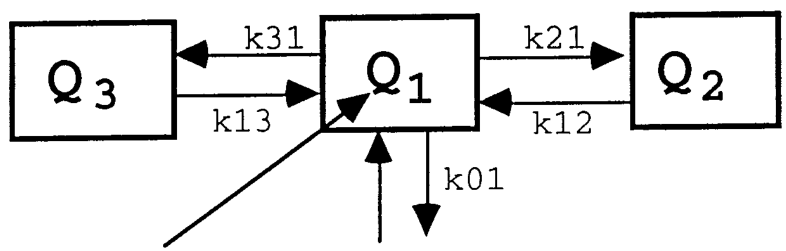


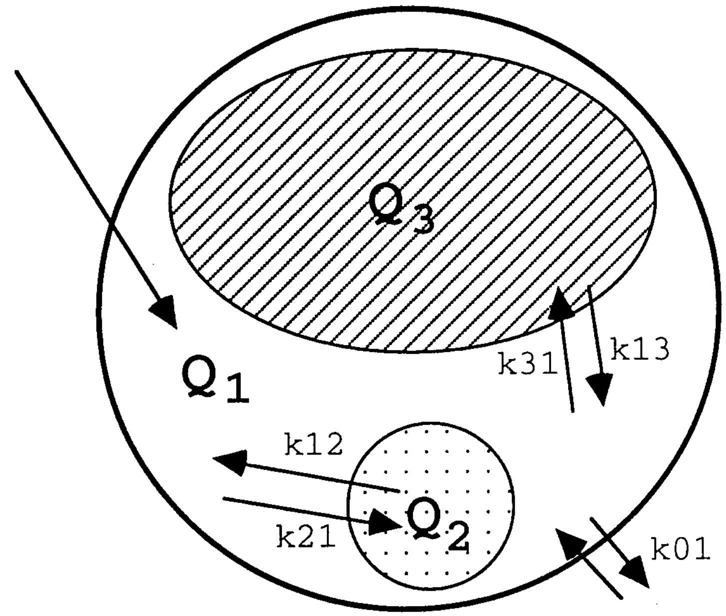
Pool 1 = Plasma zinc pool
Pool 2 includes pool 1.
Pool 3 includes pool 2.

For 1 day observation,
$g_3 = 0$. (Truncated)

Mammillary Model (Two lobes / single outlet)

i.e., a linear kinetic system which has noncentral or peripheral pools, each separately connected to a central pool without interconnection among peripheral pools.





Based on the literature;
Q_1 represents central or plasma Zn compartment
Q_2 represents metabolically active liver Zn compartment
Q_3 represents Zn compartment for liver, red cells, muscle and other tissues

Specific models utilized

Open model : the 9 day observation

No restriction in the parameters

$K_1, K_2, K_3, g_1, g_2, g_3 \Rightarrow$ *Compartmental parameters*

Closed model: the 1 day observation

Parameter restiction: $g_3 = 0$, i. e., $k_{01} = 0$

$K_1, K_2, K_3, g_1, g_2 \Rightarrow$ *Compartmental parameters*

Restricted open model: the 1 day observation

Parameter restriction: $g_3 = 0.11$ (mean of empirical values of g_3)

$K_1, K_2, K_3, g_1, g_2 \Rightarrow$ *Compartmental parameters*

Solving the mammillary model

Using Landaw et al's (1984) algorithm

$$k_{11} = k_{01} + k_{21} + k_{31} = \frac{\sum_{i=1}^{3} K_i g_i}{\sum_{i=1}^{3} K_i}$$

Roots of the numerator of Laplace transformation of tri-exponential function give $k_{22} = k_{12} + k_{21}$ and $k_{33} = k_{13} + k_{31}$. (Using Mathematica Software)

$$\gamma_j = k_{ij} k_{ji} = \frac{\sum_{i=1}^{3} K_i}{\sum_{i=1}^{3} \frac{K_i}{\left(k_{jj} - g_i\right)^2}}$$

For the single outlet or closed model, Q_2, Q_3, and 'k's are uniquely determined.

$$k_{22} = k_{12}$$

$$k_{33} = k_{13}$$

Q_1: *Plasma Zn Compartment (Central Compartment) from Extrapolation*

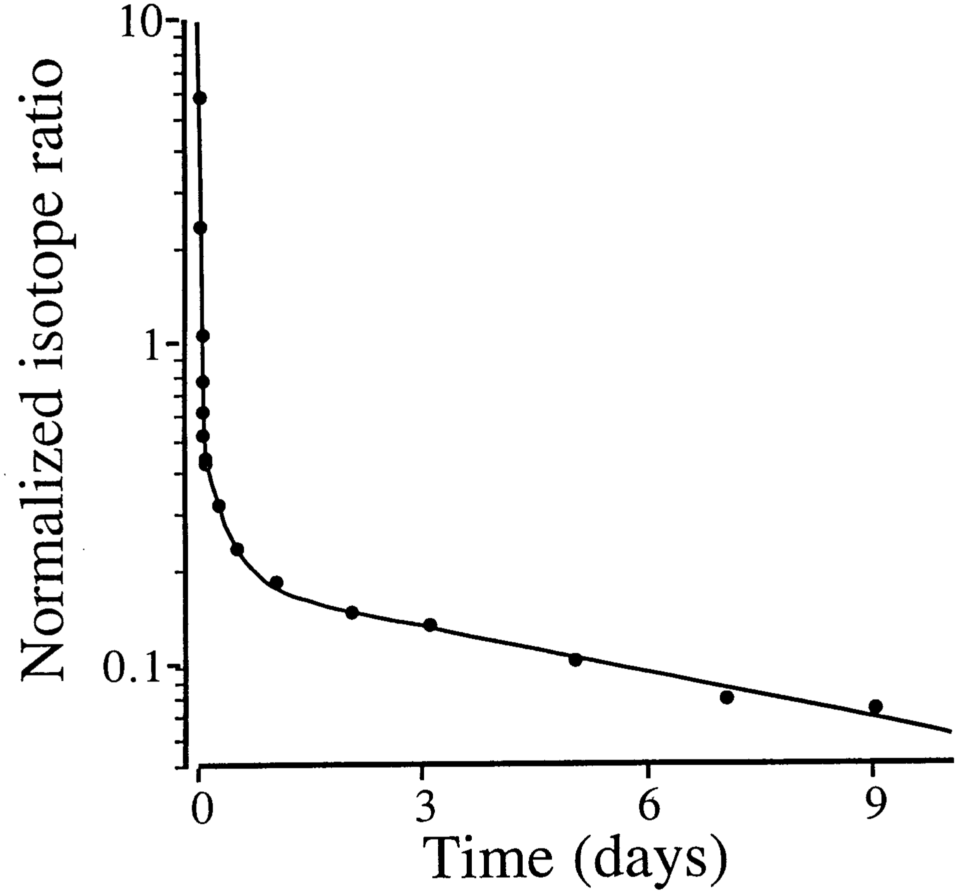
$$Q_2 = \gamma_2 / k_{12}\, Q_1$$

$$Q_3 = \gamma_3 / k_{13}\, Q_1$$

So – called Rapidly Exchanging Zn Pool (EZP) $= Q_1 + Q_2 + Q_3$

Results

Disappearance Curve with Tri-Exponential Function



Simplex minimization of residual square by nonlinear regression of SYTAT Software using the tri-exponential model

$$\textit{Logarithm of normalized isotope ratio} = Log\left(K_1 e^{-g_1} + K_2 e^{-g_2} + K_3 e^{-g_3}\right)$$

Determined coefficients and R-square for the truncated model

Subject	R^2	K_1	g_1	K_2	g_2	K_3
1	0.998	9.051	106.8	0.6328	5.260	0.2100
2	0.997	8.051	118.7	0.3732	4.823	0.1693
3	0.995	14.27	165.6	0.4190	4.086	0.2329
4	0.982	8.200	112.5	0.3461	3.147	0.1992
5	0.997	9.220	126.4	0.3627	3.747	0.1743
6	0.995	10.27	136.3	0.3309	4.222	0.2312
Mean		9.844	127.7	0.4108	4.214	0.2028
SD		2.110	19.4	0.103	0.688	0.0249
CV		21	15	25	16	12

Percent deviation of the coefficients of the truncated model from the tri-exponential model

Subject	K_1	g_1	K_2	g_2	K_3
1	2.9	5.3	10.0	38.0	9.8
2	-0.8	-1.3	2.0	-14.1	-11.5
3	3.3	3.2	3.5	44.6	9.8
4	2.0	2.8	5.9	33.8	3.3
5	0.7	0.9	5.2	6.2	-5.0
6	3.1	3.7	9.1	47.2	3.2
Mean	1.9	2.5	5.9	26.0	1.6
SD	1.5	2.1	2.9	22.4	7.7

Except g_2, the coefficients estimated from the truncated model (1 day observation) agree well with the coefficients determined from the tri-exponential model (9 day observation).

Determined coefficients and R-square for the tri-exponential model

Subject	R^2	K_1	g_1	K_2	g_2	K_3	g_3
1	0.995	8.796	101.4	0.5753	3.813	0.1912	0.1198
2	0.998	8.118	120.2	0.3659	5.612	0.1913	0.1057
3	0.996	13.82	160.5	0.4049	2.825	0.2122	0.1029
4	0.991	8.036	109.4	0.3269	2.352	0.1928	0.1236
5	0.998	9.157	125.3	0.3449	3.530	0.1834	0.1100
6	0.996	9.959	131.4	0.3032	2.868	0.2241	0.1271
Mean		9.648	124.7	0.3869	3.500	0.1992	0.1149
SD		1.975	18.8	0.0900	1.059	0.0141	0.0091
CV		20	15	23	30	7	8

Subject 3 is a woman. When Subject 3 is removed, K_1 becomes 8.813 ± 0.710 (CV 8%); g_1 117.5 ± 10.8 (CV 9 %).

K_3 and g_3 are less changeable compared to other coefficients, which enables the restricted open mammillary model.

Comparison of rapidly exchanging Zn pool (EZP) by various methods (mg)

Subject	Open mammillary	Closed mammillary	3 - 9 day plasma extrapolation	1 day plasma	3 - 9 day urine extrapolation	1 day urine	2 day urine
Interval	5 min - 9 d	5 min - 1 d	3 - 9 d	1 d	3 - 9 d	1 d	2 d
1	169	202	281	206			
2	172	213	228	206	193	147	203
3	191	244	242	233	250	195	237
4	166	194	261	195	198	145	198
5	196	251		256			260
6	162	182	218	182	190	158	219
Mean	176	214	246	213	208	161	223
SD	14	28	25	27	28	23	26
CV	8	13	10	13	14	14	11

Assuming the open mammillary model gives the 'true' EZP as proposed by Miller (1994), overestimation of EZP by closed mammillary model, pool 3 of 'concentric design', the 3 - 9 d plasma NIR extrapolation and 1 day plasma is obvious. The restricted open mammillary model corrected the overestimation of EZP to some extent.

R-squares between EZP from the open mammillary model and from others are as follows:

Closed mammillary model,	0.967 ($p = 0.0004$)
Restricted open mammillary model,	0.944 ($p = 0.001$)
Pool 3 of the concentric design,	0.681 ($p = 0.043$)
The 3 - 9 day plasma extrapolation,	0.042 ($p = 0.741$)
One day plasma pool,	0.918 ($p = 0.0026$)

One day plasma pool gave a good approximation of EZP unless it was based on just one day NIR.

Comparison of the sizes (mg) of the compartments Q_1, Q_2, Q_3 determined from open (O), closed (C) and restricted open (R) mammillary models

	Q_1			Q_2			Q_3		
Subject	**O**	**C**	**R**	**O**	**C**	**R**	**O**	**C**	**R**
1	4.44	4.29	4.26	25.4	19.7	18.1	139	178	129
2	4.89	4.94	4.89	26.3	29.8	26.2	161	216	160
3	2.94	2.85	2.82	37.4	29.0	25.9	122	150	111
4	4.96	4.85	4.81	47.6	39.8	35.3	119	168	117
5	4.38	4.35	4.34	37.4	35.3	33.3	149	204	144
6	4.05	3.92	3.86	43.7	32.8	26.4	108	147	114

O: Open mammillary model from 0 - 9 days
C: Closed mammillary model from 0 - 1 day
R: Restricted open mammillary model from 0 - 1 day

Exact Q_1 and Q_3 from open mammillary model (0 - 9 days) are well estimated from 1 day observation using the closed or restricted open mammillary model. Overestimation of Q_3 found in the closed mammillary model was corrected by the restricted open mammillary model.

Summary

1. Plasma Zn kinetics can be analyzed using ^{67}Zn as tracer and Quadrapole ICP-MS as a detector. The observed values of the coefficients were similar to the values found in Wastney's (1986) data and Miller's (1994) model.

2. Some important kinetic parameters derived from the 9 day observation interval using a tri-exponential curve fitting and an open mammillary model are predictable from the 1 day observation using a truncated model and a closed mammillary model. Predictable parameters with acceptable accuracy are K_1, K_2, K_3, g_1, so-called 'rapidly' exchanging Zn pool (EZP), Q_1 (central or plasma Zn compartment), Q_3 (larger peripheral compartment) and k_{11} (sum of the fractional rate constant of central compartment).

3. When g_3 is set at 0.11 to analyze within 1 day data (restricted open mammillary model), overestimation of Q_2, Q_3 and EZP can be corrected to some extent.

4. One day plasma Zn pool is a good estimate of EZP rather than a pool size determined from 3 - 9 day plasma extrapolation. Normalized isotope ratios of 0.07 to 0.14 after 3 to 9 days did not allow the extrapolation to predict an accurate EZP because the CV of the isotope ratio measurement was 0.5 to 1.0 %.

Acknowledgment

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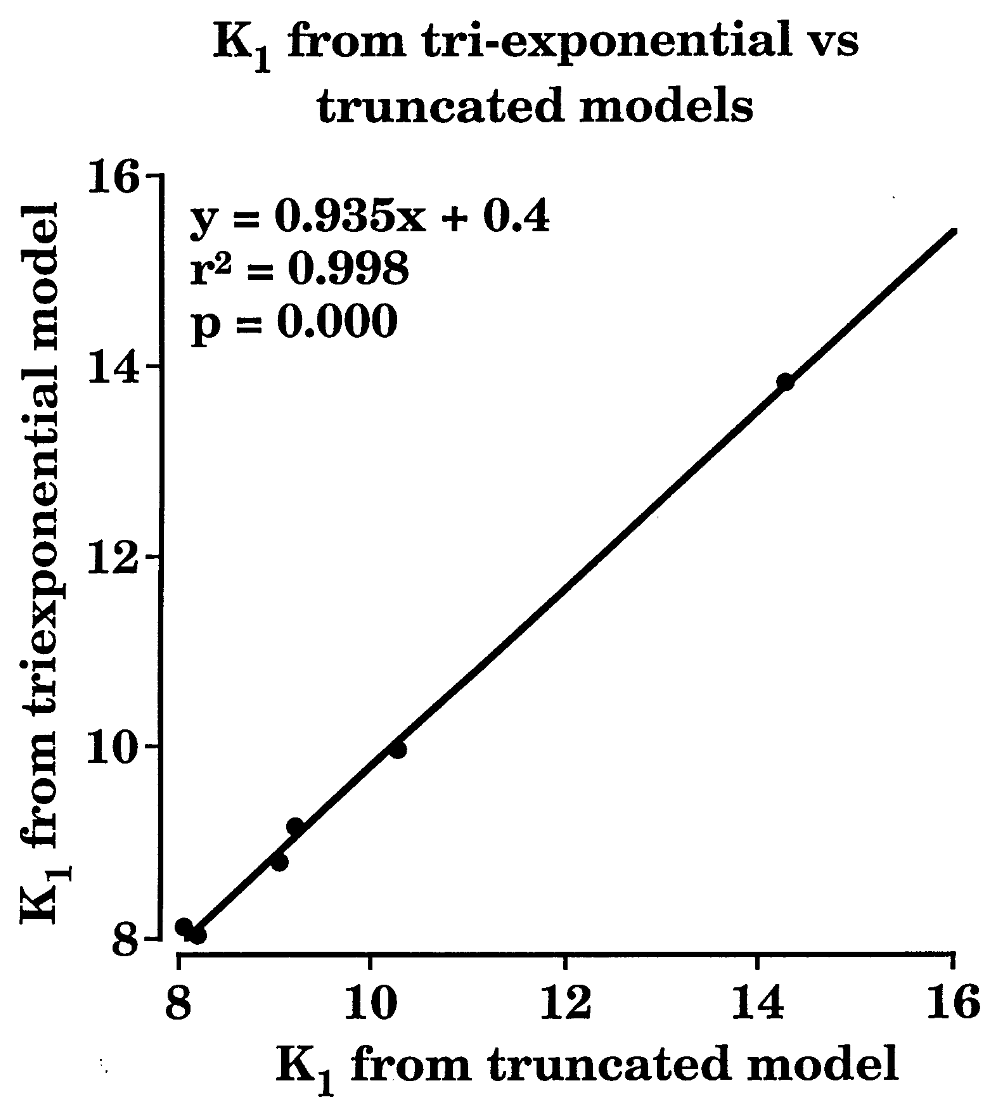
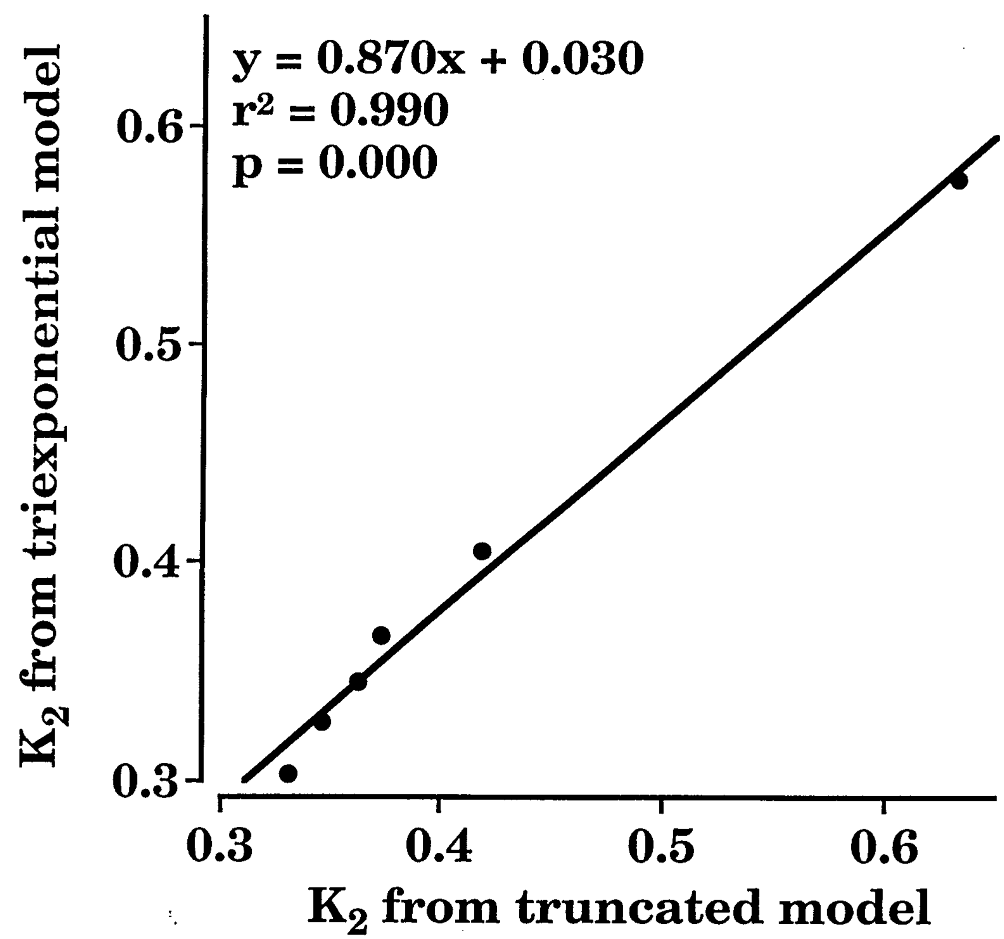
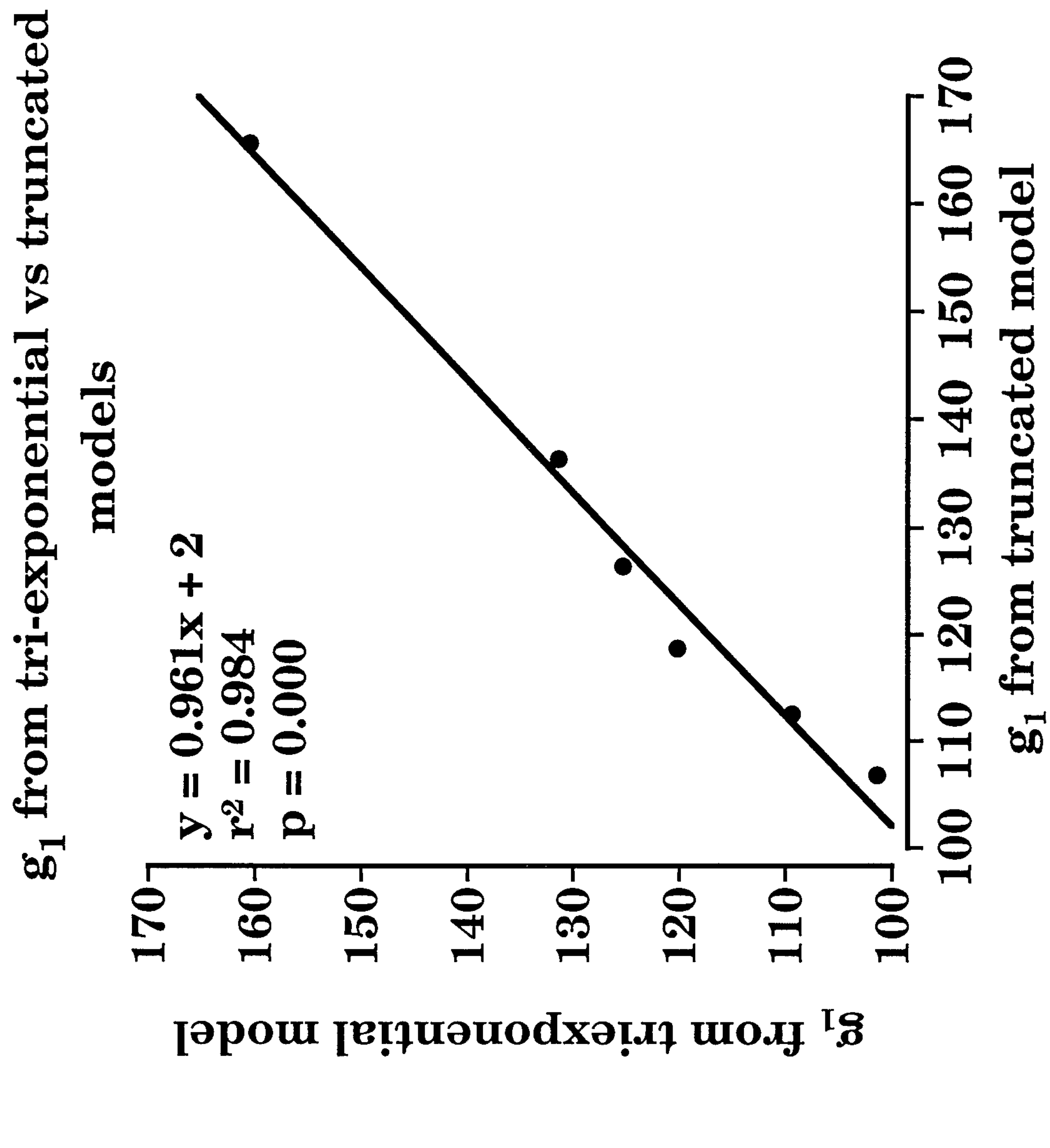

K1 from tri-exponential vs
truncated models
y = 0.935x + 0.4
r² = 0.998
p = 0.000
K1 from triexponential model
K1 from truncated model
8
10
12
14
16

K_2 from tri-exponential vs truncated model





g1 from tri-exponential vs truncated models
y = 0.961x + 2
r2 = 0.984
p = 0.000
g1 from triexponential model
g1 from truncated model
100
110
120
130
140
150
160
170

A Convenient Approach to Determine the Rapidly Exchangeable Zinc Pool in Humans.

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Abstract

Current clinical indices for evaluating Zn status are imperfect. Prasad et al (1963) used the plasma Zn disappearance to show that growth stunted adolescents were low in Zn. Miller et al (1994) found that the so-called "rapidly exchangeable" Zn pool size (EZP) was related to dietary Zn using stable Zn isotopes and fast atom bombardment mass spectrometry (FAB MS). We therefore compared EZP after i.v. ^{67}Zn derived from 0 - 9 days and from 0 - 24 hours plasma collections or spot urine collections in fourteen subjects (5 men and 9 women; age men 41 ± 15 y, women 27 ± 5; BMI men 26.1 ± 3.0, women 20.9 ± 1.4). Plasma Zn isotope ratios (IR) were measured by inductively coupled plasma - mass spectrometry (ICP-MS). The experimental IR was corrected for the baseline and divided by the natural Zn IR to give the normalized IR (NIR). EZP was determined from plasma obtained 24 hr and from 3-9 days, and a spot urine sample obtained day 1 and 2 and from 3 - 9 days after iv dose of 2 mg ^{67}Zn given to the subjects. Calculated pool sizes from the closed mammillary model, 3-9 day urine, or 24 h plasma and 2 day spot urine were similar, indicating a consistent overestimation (20 %) compared to a norm for EZP derived from the open mammillary model. Good correlation was found between EZP and 24 h urinary creatinine excretion.

Introduction

Biochemical indices for evaluating Zn status are imperfect and the specificity of physiological indices are unknown. Zn kinetic parameters have been measured using radioactive Zn. Prasad et al (1963) used ^{65}Zn to show rapid disappearance of plasma Zn in growth stunted adolescents. Using ^{69m}Zn and ^{65}Zn Aamodt et al (1979, 1982) observed altered Zn kinetics after oral loading of 100 mg Zn. Foster et al (1984) and Wastney et al (1986) developed an integrated Zn kinetic model using ^{65}Zn and ^{69m}Zn.

Stable Zn isotopes are alternatives. Wastney et al (1991) compared the results obtained from ^{65}Zn and ^{70}Zn using neutron activation analysis. Miller et al (1991, 1994) used stable Zn isotopes and FABMS for measuring Zn pools. Using Quadrapole ICP-MS, Lowe et al (1992) analyzed the 120 min kinetics with ^{70}Zn and Yokoi et al (1992, 1994a, 1994b) evaluated Zn disappearance from 30 to 60 min after iv dose of ^{67}Zn. Fairweather-Tait et al (1993) measured Zn pools using ^{70}Zn and thermal ionization mass spectrometry (TIMS). Scott and Turnlund (1994) adapted Wastney's approach (1986) to ^{67}Zn and ^{70}Zn tracer using TIMS.

Two alternative methods for measurement of Zn pool sizes are possible: the mathematical approaches, i.e., the deconvolution method for subsystem (Foster 1979, 1984; Wastney 1986) or the mammillary compartment model; and the approximation from linear regression in semilogarithmic plots as proposed by Miller et al (1994) or spot plasma and urine data . We therefore compared Zn pool sizes obtained from the mathematical approach (the mammillary compartment model) and the approximation method.

Objective

- To develop a simple method for determination of rapidly exchangeable zinc pool sizes (EZP)
- To compare EZP and 24 h urinary creatinine excretion

Definition of EZP (rapidly exchangeable Zn pool size)

There is no established clear definition of EZP. Foster et al (1979) and Wastney et al (1986) have identified several compartments that exchange "completely" with plasma Zn within a 2-d period. Therefore EZP is the sum of pools that reach more than 95 % of pseudoequilibrium with the central, so-called plasma Zn compartment, at 2 days after the iv dose of the tracer. In practice, EZP is the sum of pools that have a sum of fractional rate constants larger than 0.8 day^{-1} in the mammillary model.

Method

1. Inject 2 mg ^{67}Zn intravenously to human subjects and collect blood samples at baseline (before the administration) and 5, 15, 30, 40, 50, 60, 90 minutes, 2, 6, 12 hours, 1, (2), 3, 5, 7, 9 days later and spot urine samples 1, 2, 3, 4, 5, 6, 7, 8 and 9 days later.

2. Digest the specimens, extract Zn using diethylammonium diethyldithiocarbamate (chelator) and carbon tetrachloride (organic solvent) and measure the Zn isotope ratio (^{67}Zn:^{64}Zn, ^{67}Zn:^{66}Zn, ^{67}Zn:^{68}Zn, ^{67}Zn:^{70}Zn) by ICP-MS (Plasma Quad, VG Instruments) based on Yokoi et al (1994a; 1994b).

3. Subtract the baseline from the measured isotope ratio and divide the ratio by natural Zn isotope ratio to obtain the normalized isotope ratio (NIR).

4. Calculate EZP as a norm using the open mammillary model (Landaw et al 1984) from the obtained coefficients of the polyexponential function fitted to 5 min - 9 day plasma data.

5. Calculate approximation of EZP from 5 min - 24 h plasma data using the closed mammillary model, from the extrapolation of logarithm of NIR of 3-9 day plasma or urine, and from NIR of the spot 24 hr plasma and 1 or 2 day spot urine.

6. Compare EZP and 24 hr urinary creatinine excretion.

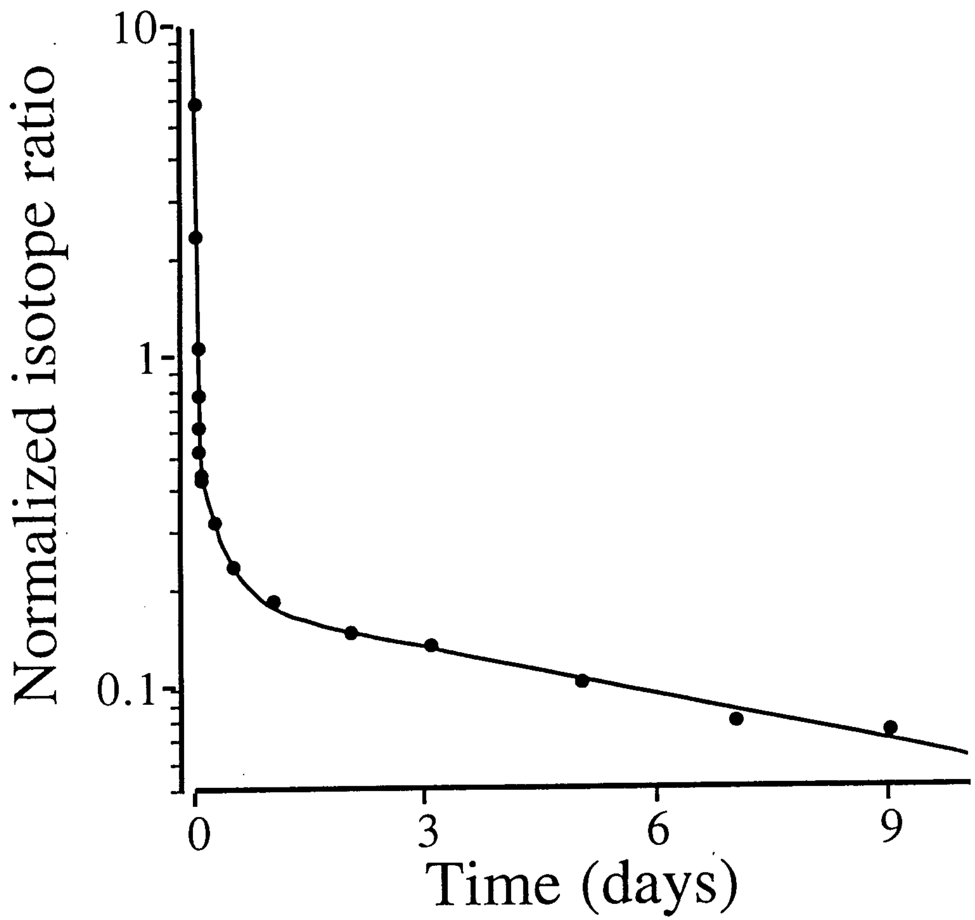
Characteristics of the subjects

	Age (years)	Body weight (kg)	Body height (m)	Body mass index	Lean body weight (kg)	Plasma Zn (ng/ml)	Plasma Zn binding capacity (ng/ml)
5 Males							
Mean	**41.4**	**80.5**	**1.76**	**26.1**	**62.0**	**928**	**5979**
SD	**14.9**	**8.5**	**0.06**	**3.0**	**4.3**	**89**	**272**
9 Females							
Mean	**27.1**	**59.0**	**1.68**	**20.9**	**44.8**	**828**	**5498**
SD	**5.2**	**6.9**	**0.05**	**1.4**	**4.3**	**84**	**565**

Five male and one female subjects attended the 9 day observation study.

Results

Disappearance Curve with Tri-Exponential Function



Simplex minimization of residual square by nonlinear regression of SYTAT Software using the tri-exponential model

$$\textit{Logarithm of normalized isotope ratio} = Log\left(K_1 e^{-g_1 t} + K_2 e^{-g_2 t} + K_3 e^{-g_3 t}\right)$$

Percent deviation from the open mammillary model

Subject	Closed mammillary	3 - 9 day plasma extrapolation	1 day plasma	3 - 9 day urine extrapolation	1 day urine	2 day urine
Interval	5 min - 1 d	3 - 9 d	1 d	3 - 9 d	1 d	2 d
1	**20**	**67**	**22**			
2	**24**	**33**	**20**	**12**	**- 14**	**18**
3	**28**	**27**	**22**	**31**	**2**	**24**
4	**17**	**57**	**18**	**19**	**- 13**	**19**
5	**28**		**30**			**32**
6	**12**	**34**	**12**	**17**	**- 3**	**35**
Mean	**21**	**44**	**21**	**20**	**- 7**	**26**
SD	**6**	**17**	**6**	**8**	**8**	**8**

Assuming the open mammillary model gives the 'true' EZP as proposed by Miller (1994), overestimation of EZP were found by various approximation methods.
The closed mammillary model and 24 h plasma pool gave a good approximation of EZP.

Comparison of rapidly exchanging Zn pool (EZP) by various methods (mg)

Subject	Open mammillary	Closed mammillary (= truncated)	Retricted open mammillary	Pool 3 of 'concentric design'	3 - 9 day plasma extrapolation	1 day plasma
Interval	5 min - 9 d	5 min - 1 d	5 min - 1 d	5 min - 9 d	3 - 9 d	1 d
1	**169**	**202**	**151**	**222**	**281**	**206**
2	**192**	**251**	**191**	**222**	**-**	**256**
3	**162**	**182**	**140**	**200**	**218**	**182**
4	**171**	**213**	**157**	**220**	**228**	**206**
5	**191**	**244**	**182**	**231**	**242**	**233**
6	**156**	**184**	**144**	**203**	**233**	**185**
Mean	**174**	**213**	**161**	**216**	**240**	**211**
SD	**14**	**27**	**19**	**11**	**22**	**26**
CV	**8**	**13**	**12**	**5**	**9**	**12**

Percent deviation from the open mammillary model

Subject	Closed mammillary (= truncated)	Restricted open mammillary	Pool 3 of 'concentric design'	3 - 9 day plasma extrapolation	1 day plasma pool
1	**19.8**	**-10.2**	**31.6**	**66.5**	**22.1**
2	**30.3**	**-0.7**	**15.4**	**-**	**33.0**
3	**12.2**	**-14.0**	**23.1**	**34.2**	**12.0**
4	**24.4**	**-8.2**	**28.4**	**33.0**	**20.2**
5	**27.6**	**-4.7**	**21.0**	**26.8**	**22.1**
6	**17.6**	**-7.9**	**30.0**	**49.2**	**18.5**
Mean	**22.0**	**-7.6**	**24.9**	**42.0**	**21.3**
SD	**6.1**	**4.2**	**5.7**	**14.3**	**6.2**

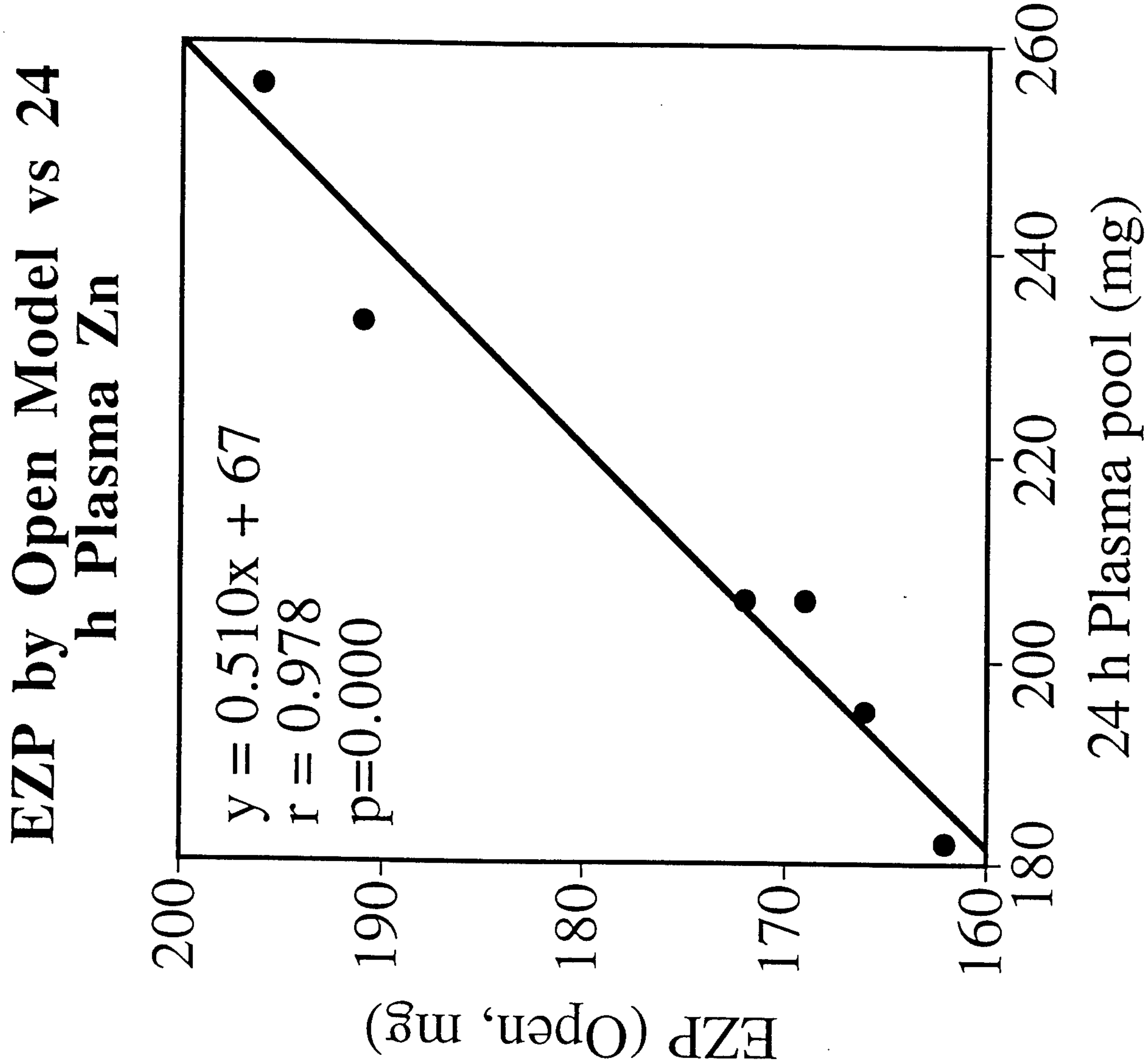
Summary

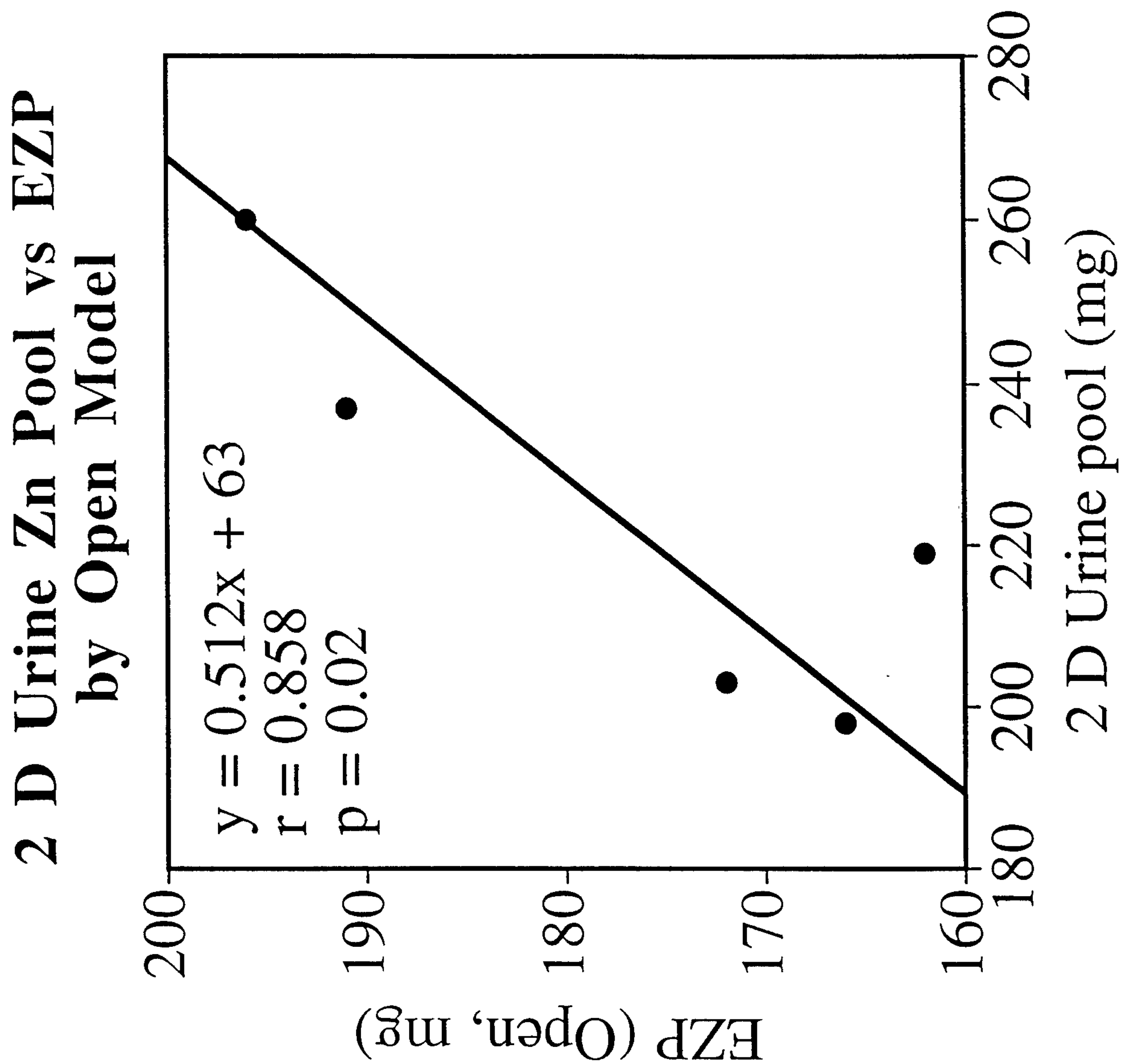
1. Plasma Zn kinetics can be analyzed using ^{67}Zn as tracer and Quadrapole ICP-MS as a detector. The observed values of the coefficients were similar to the values found in Wastney's (1986) data and Miller's (1994) model.

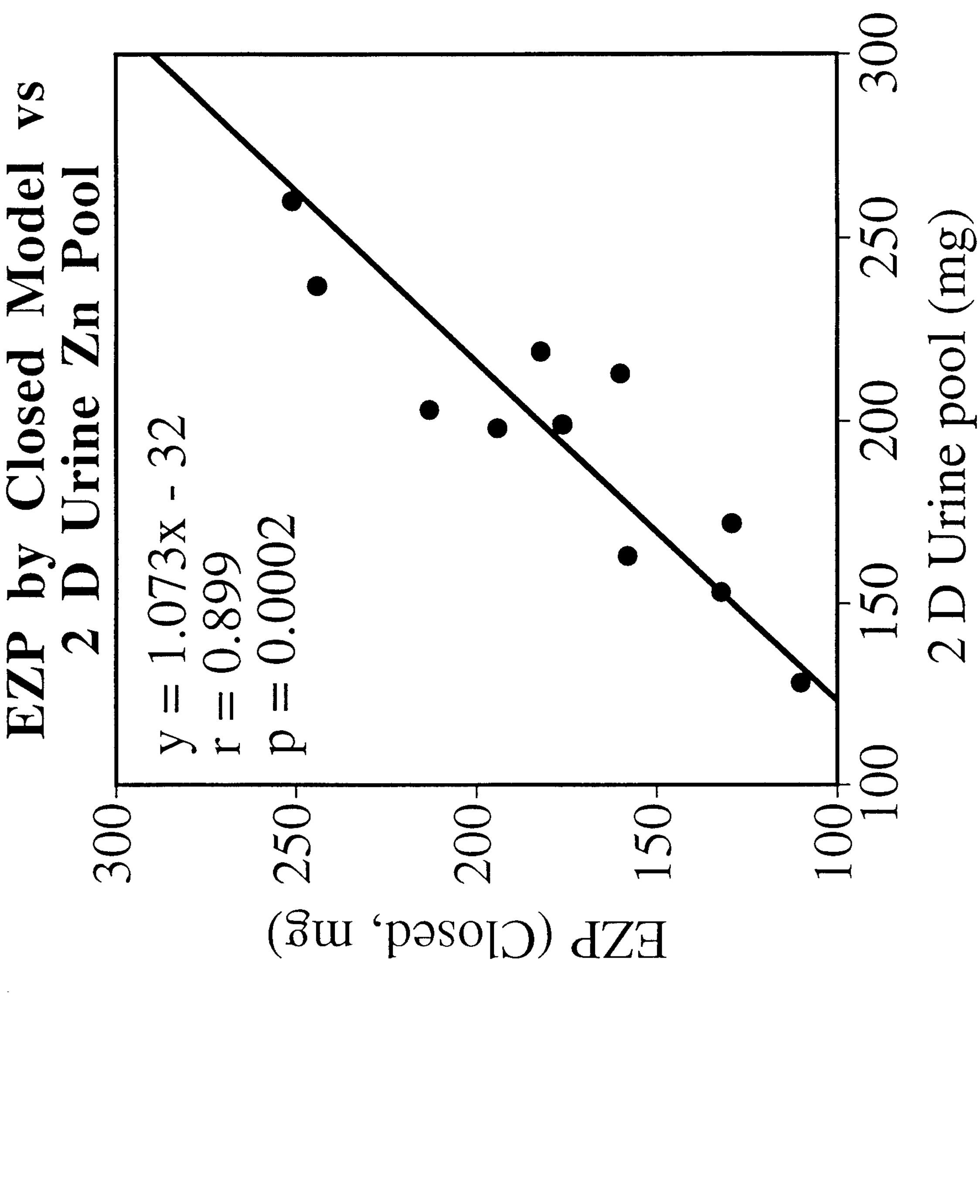
2. Some important kinetic parameters derived from the 9 day observation interval using a tri-exponential curve fitting and an open mammillary model are predictable from the 1 day observation using a truncated model and a closed mammillary model. Predictable parameters with acceptable accuracy are K_1, K_2, K_3, g_1, so-called 'rapidly' exchanging Zn pool (EZP), Q_1 (central or plasma Zn compartment), Q_3 (larger peripheral compartment) and k_{11} (sum of the fractional rate constant of central compartment).

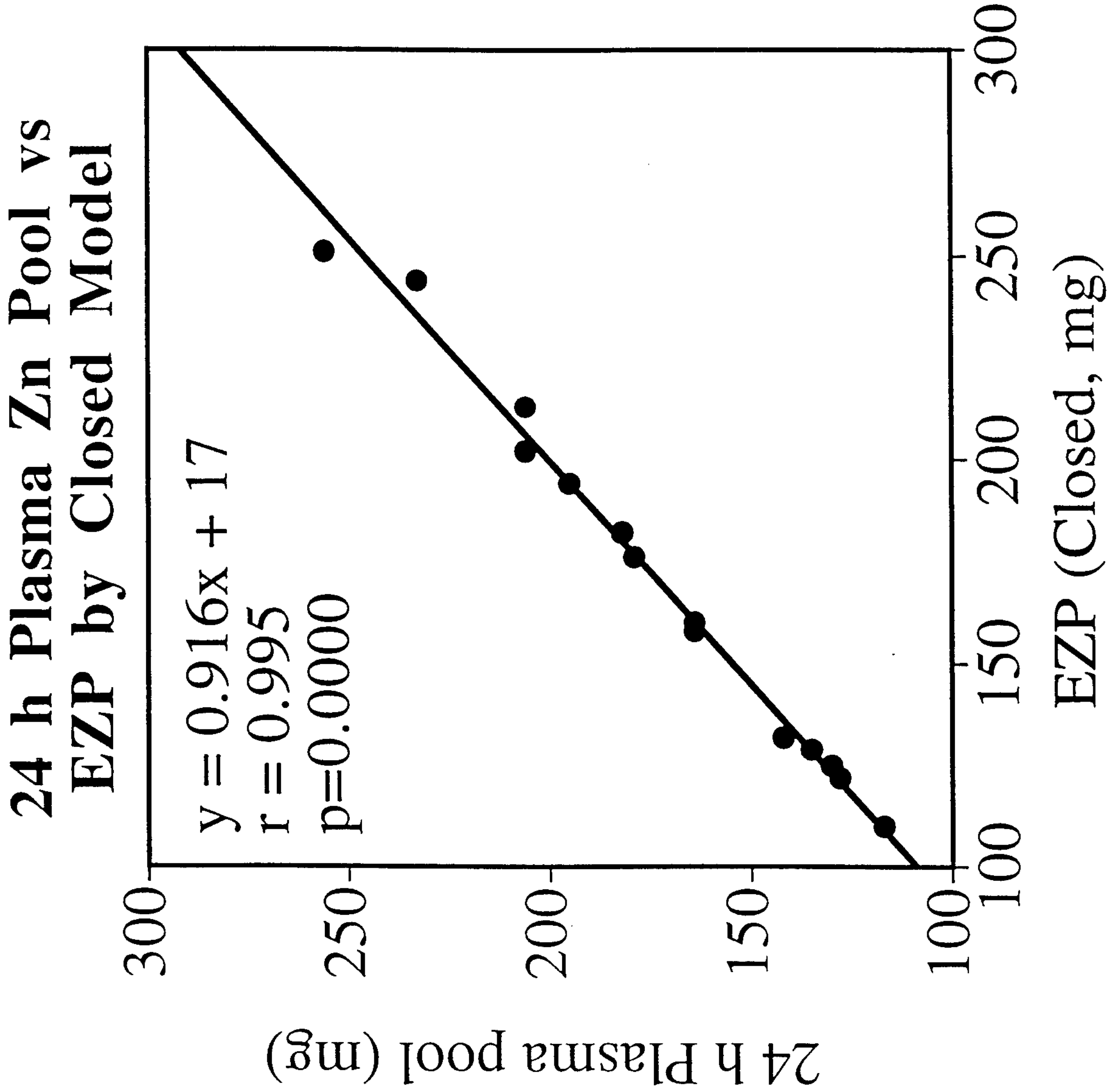
3. When g_3 is set at 0.11 to analyze within 1 day data (restricted open mammillary model), overestimation of Q_2, Q_3 and EZP can be corrected to some extent.

4. One day plasma Zn pool is a good estimate of EZP rather than a pool size determined from 3 - 9 day plasma extrapolation. Normalized isotope ratios of 0.07 to 0.14 after 3 to 9 days did not allow the extrapolation to predict an accurate EZP because the CV of the isotope ratio measurement was 0.5 to 1.0 %.

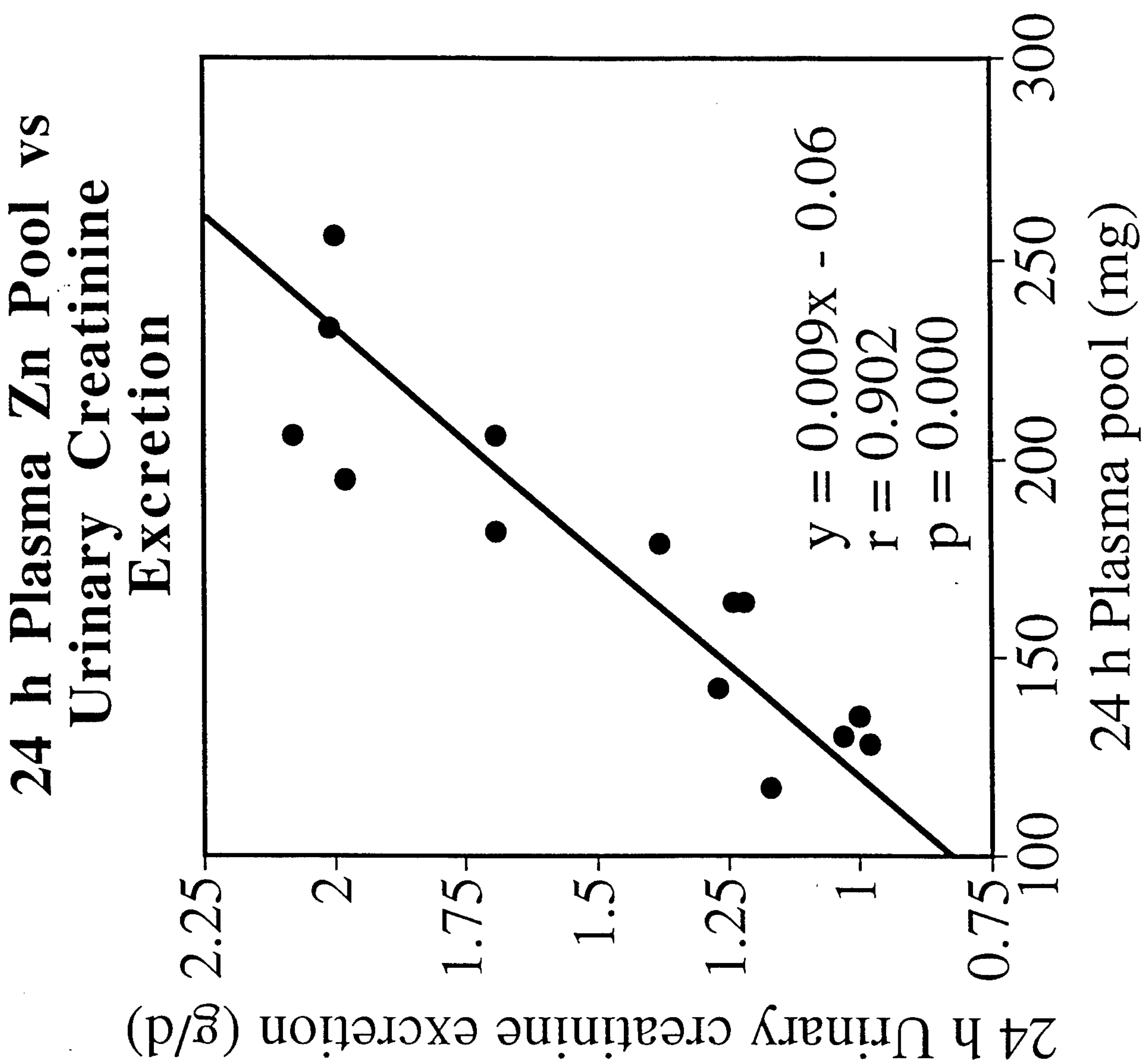

EZP by Open Model vs 24 h Plasma Zn
y = 0.510x + 67
r = 0.978
p=0.000
EZP (Open, mg)
200
190
180
170
160
180
200
220
240
260
24 h Plasma pool (mg)


2 D Urine Zn Pool vs EZP
by Open Model
y = 0.512x + 63
r = 0.858
p = 0.02
EZP (Open, mg)
200
190
180
170
160
180
200
220
240
260
280
2 D Urine pool (mg)


EZP by Closed Model vs
2 D Urine Zn Pool
y = 1.073x - 32
r = 0.899
p = 0.0002
EZP (Closed, mg)
300
250
200
150
100
2 D Urine pool (mg)
100
150
200
250
300



24 h Plasma Zn Pool vs
EZP by Closed Model
y = 0.916x + 17
r = 0.995
p=0.0000
24 h Plasma pool (mg)
EZP (Closed, mg)
100
150
200
250
300


24 h Plasma Zn Pool vs Urinary Creatinine Excretion
y = 0.009x - 0.06
r = 0.902
p = 0.000
24 h Plasma pool (mg)
24 h Urinary creatinine excretion (g/d)
100
150
200
250
300
0.75
1
1.25
1.5
1.75
2
2.25

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